JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. I · WASHINGTON, D. C., JANUARY 10, 1914 · NO. 4

# ENVIRONMENTAL INFLUENCES ON THE PHYSICAL AND CHEMICAL CHARACTERISTICS OF WHEAT

By J. A. LE CLERC, *Chief*, and P. A. YODER, *Assistant Chemist*,
*Plant-Chemistry Laboratory, Bureau of Chemistry*

## INTRODUCTION

A former series of experiments [1] conducted in the Bureau of Chemistry showed that neither the composition nor the physical characteristics of wheat are to any great extent hereditary. The protein, gluten, and ash contents, as well as the size of the berry, the weight of a bushel, and the flintiness of the kernel, were found to be dependent upon the climatic conditions prevailing during the growing period of the plant. Seed of Kansas wheat containing 20 per cent of protein and showing 100 per cent of flinty kernels and seed of California wheat containing 10 per cent of protein with 13 per cent of flinty kernels when grown side by side in South Dakota yielded crops of identical composition and physical appearance. The same was true of these Kansas and California seeds when grown in California. The crops grown in California were, however, entirely unlike those grown in South Dakota, owing to the great difference in climatic conditions. It was shown in a most conclusive manner that environment plays a major part in influencing both the chemical composition and the physical appearance of a wheat crop. Cropping through a number of generations under widely different environments therefore does not alter permanently or make a noticeable impression upon the transmissible physical and chemical properties of wheat.

Similar experiments, involving the transference of soil, are reported by Shaw and Walters.[2] In the main, their observations, based on crops grown throughout a period of three years in one locality, harmonize with the conclusions here presented, which are founded on the wider range of experimental data now at hand, involving crops grown for four years on three different types of soil in three different localities having widely

[1] Le Clerc, J. A., and Leavitt, Sherman. Tri-local experiments on the influence of environment on the composition of wheat. U. S. Dept. Agr., Bur. Chem. Bul. 128, 18 p., 1910.

[2] Shaw, G. W., and Walters, E. H. A progress report upon soil and climatic factors influencing the composition of wheat. Cal. Agr. Exp. Sta. Bul. 216, p. 549-574, 1911.



17073°—14——1

varying climatic conditions. In some particulars, however, the conclusions which seemed justifiable from their experiments are not borne out by these more extensive data.

The experiments discussed in this article were designed to study further the environmental influences and to show the rôle exerted by the soil and the part played by climatic conditions, such as rainfall, sunshine, humidity of the atmosphere, temperature, winds, and elevation above sea level. As in the case of the previous experiments,[1] they were carried on in cooperation with the Office of Cereal Investigations of the Bureau of Plant Industry. The agricultural experiment stations of Maryland, Kansas, and California cooperated by growing the crops.

## CONDUCT OF THE EXPERIMENTS

In order to distinguish between the rôle played by soil and that by environment other than soil, samples of soil were interchanged among three localities, Maryland (College Park), Kansas (Hays), and California (Davis), which differ widely in climatic conditions. From each locality sections of a normally fertile wheat-producing soil 5 feet square and 3 feet deep were dug up in 3-inch layers, sacked, and replaced in the same original position. To obviate any differences due to this manipulation a portion of soil 5 feet square and 3 feet deep from each locality was likewise dug up in 3-inch layers, sacked, and stored until the soils from the two other localities had arrived, when all three samples were placed in their respective positions. A fourth plat of soil of the same size was allowed to remain undisturbed in each locality to determine whether the treatment to which the three other soils had been subjected would exert any influence on the composition of the grain. Thus, there were 12 experimental plats, 4 in each locality, as shown in the following plan:

TWELVE EXPERIMENTAL PLATS

California:
- Plat of undisturbed California soil, or check plat.
- Plat of disturbed California soil.
- Plat of Kansas soil.
- Plat of Maryland soil.

(Each taken up in 3-inch layers and replaced in original order.)

Kansas:
- Plat of undisturbed Kansas soil, or check plat.
- Plat of disturbed Kansas soil.
- Plat of California soil.
- Plat of Maryland soil.

(Each taken up in 3-inch layers and replaced in original order.)

Maryland:
- Plat of undisturbed Maryland soil, or check plat.
- Plat of disturbed Maryland soil.
- Plat of California soil.
- Plat of Kansas soil.

(Each taken up in 3-inch layers and replaced in original order.)

[1] Le Clerc and Leavitt. Op. cit.

During the first two years, 1908 and 1909, Crimean wheat obtained from seed grown in Kansas was used on all 12 plats. As this variety was not adapted to conditions prevailing in Maryland and California, Turkey wheat was selected for 1910, 1911, and 1912. The change from Crimean to Turkey wheat did not interfere, however, with the object of the experiment, which was to determine the influence exerted by climatic conditions and soil on the composition of the crop.

The following determinations were made according to the methods given in Bulletin 107, Revised, of the Bureau of Chemistry, entitled "Official and Provisional Methods of Analysis."

Water; weight of 1,000 grains; weight of a bushel; flinty grains; nitrogen; alcohol-soluble nitrogen; fat; fiber; pentosans; sugars; ash; phosphoric acid; and potash. The alcohol-soluble nitrogen was determined by treating a certain quantity of ground wheat with a 70 per cent solution of alcohol at ordinary temperature, with frequent shaking, for several hours, and then allowing the solution to stand overnight. An aliquot part was taken and the nitrogen therein determined. The amount of nitrogen thus obtained divided by the total quantity of nitrogen in the sample gave the gliadin number.

## TABULATION OF DATA

The data are collected in a number of tables. In Table I, first column, is given the analysis of the original seed grown in Kansas in 1908, which was used as seed on all the plats for the following year's crop. The other analyses in Table I and the data in Tables II to IV were obtained on crops grown in 1909, 1910, 1911, and 1912, the results being grouped by locality. The data from the different soil plats and the check-soil plat in each locality are arranged in adjacent columns in Table I. In Table II the same data, exclusive of check-plat data, are rearranged, the results from the same soils being grouped in adjacent columns. Averages derived from these data are given in Tables III, IV, and V. In Table III are shown the averages of all the constituents from crops grown in California, Kansas, and Maryland, not including the check-soil plat, throughout the four years of the experiment. Table IV gives the averages obtained from data on the crops grown on the soils of California, Kansas, and Maryland for each of the three localities and for all four years. Finally, in Table V are shown the averages for the undisturbed or check-soil plats and for the corresponding plats in which the soil had been taken up in 3-inch layers and replaced.

TABLE I.—*Composition of wheat grown on different plats of soil in California, in Kansas, and in Maryland in 1909, 1910, 1911, and 1912.*

CRIMEAN WHEAT.

Original seed and 1909 crop.

| Determination. | Original seed grown in Kansas in 1908. | Wheat grown in California on— | | | | Wheat grown in Kansas[1] on— | | | | Wheat grown in Maryland on— | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | California check soil. | California soil. | Kansas soil. | Maryland soil. | California soil. | Kansas soil. | Kansas check soil. | Maryland soil. | California soil. | Kansas soil. | Maryland soil. | Maryland check soil. |
| Physical properties: | | | | | | | | | | | | | |
| Water.....per cent.. | 9.20 | 9.64 | 8.98 | 9.00 | 8.88 | ..... | ..... | ..... | ..... | 9.50 | 9.48 | 9.22 | ..... |
| Weight per 1,000 grains, grams..... | 26.3 | 36.2 | 34.6 | 36.4 | 25.4 | ..... | ..... | ..... | ..... | 21.2 | 23.0 | 22.2 | ..... |
| Weight per bushel..pounds.. | 57.7 | 62.7 | 61.5 | 61.5 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| Flinty grains.....per cent.. | ..... | 100 | 100 | 75 | 97 | ..... | ..... | ..... | ..... | 85 | 80 | ..... | ..... |
| On water-free basis: | | | | | | | | | | | | | |
| Nitrogen.....do.... | 2.58 | 2.59 | 2.78 | 2.01 | 2.03 | ..... | ..... | ..... | ..... | 2.69 | 2.57 | 2.38 | ..... |
| Protein (N×5.7).....do.... | 14.75 | 14.76 | 15.84 | 11.46 | 11.57 | ..... | ..... | ..... | ..... | 15.33 | 14.65 | 13.34 | ..... |
| Alcohol-soluble nitrogen, per cent..... | 1.03 | 1.23 | 1.16 | .82 | .71 | ..... | ..... | ..... | ..... | 1.10 | 1.05 | .92 | ..... |
| Gliadin in protein..per cent.. | 40 | 46 | 41 | 41 | 35 | ..... | ..... | ..... | ..... | 41 | 41 | 40 | ..... |
| Fat.....do..... | ..... | 1.67 | 1.82 | 1.82 | 1.84 | ..... | ..... | ..... | ..... | 2.16 | 2.05 | 2.15 | ..... |
| Fiber.....do..... | ..... | 2.18 | 2.33 | 2.43 | 2.39 | ..... | ..... | ..... | ..... | 2.69 | 2.62 | 2.59 | ..... |
| Pentosans.....do.... | 8.70 | 8.22 | 8.49 | 8.26 | 8.53 | ..... | ..... | ..... | ..... | 8.37 | 8.31 | 9.01 | ..... |
| Sugars.....do.... | 2.52 | 3.53 | 3.21 | 3.73 | 3.26 | ..... | ..... | ..... | ..... | 2.89 | 2.64 | 2.82 | ..... |
| Ash.....do.... | 2.05 | 1.72 | 1.63 | 1.63 | 1.90 | ..... | ..... | ..... | ..... | 2.39 | 2.30 | 2.09 | ..... |
| Phosphoric acid.....do.... | .96 | .79 | .68 | .79 | .89 | ..... | ..... | ..... | ..... | 1.23 | 1.18 | ..... | ..... |
| Potash.....do.... | .55 | .48 | .45 | .46 | .56 | ..... | ..... | ..... | ..... | ..... | .63 | ..... | ..... |
| Phosphoric acid in ash, per cent..... | 46 | 46 | 42 | 43 | 47 | ..... | ..... | ..... | ..... | 51 | 51 | ..... | ..... |
| Potash in ash.....per cent.. | 25 | 28 | 28 | 29 | 30 | ..... | ..... | ..... | ..... | ..... | 27 | ..... | ..... |

TURKEY WHEAT.

1910 crop.

| Determination. | Wheat grown in California on— | | | | Wheat grown in Kansas on— | | | | Wheat grown in Maryland on— | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | California check soil. | California soil. | Kansas soil. | Maryland soil. | California soil. | Kansas soil. | Kansas check soil. | Maryland soil. | California soil. | Kansas soil. | Maryland soil. | Maryland check soil. |
| Physical properties: | | | | | | | | | | | | |
| Water.....per cent.. | 9.87 | 9.68 | 9.67 | 8.99 | 9.39 | 9.03 | 9.30 | 9.12 | 9.00 | 10.66 | 9.73 | ..... |
| Weight per 1,000 grains.....grams.. | 31.2 | 28.3 | 34.3 | 21.5 | 26.1 | 22.6 | 23.3 | 24.0 | 28.0 | 31.5 | 25.9 | ..... |
| Weight per bushel.....pounds.. | 60.5 | ..... | 61.8 | ..... | 58.3 | 56.9 | 57.2 | 55.8 | ..... | 57.7 | ..... | ..... |
| Flinty grains.....per cent.. | 99 | 100 | 70 | 100 | 99 | 100 | 100 | 100 | 0 | 0 | 0 | ..... |
| On water-free basis: | | | | | | | | | | | | |
| Nitrogen.....do.... | 2.16 | 2.39 | 1.86 | 2.86 | 2.80 | 3.28 | 3.23 | 3.12 | 1.80 | 1.90 | 2.05 | ..... |
| Protein (N×5.7).....do.... | 12.31 | 13.63 | 10.60 | 16.28 | 15.98 | 18.73 | 18.43 | 17.81 | 10.27 | 10.84 | 11.68 | ..... |
| Alcohol-soluble nitrogen.....do.... | ..... | .90 | 1.05 | .74 | ..... | 1.23 | 1.44 | 1.32 | 1.29 | ..... | .75 | ..... |
| Gliadin in protein.....do.... | 44 | 44 | 40 | ..... | 44 | 41 | 41 | 41 | ..... | 39 | ..... | ..... |
| Fat.....do.... | 2.01 | 2.13 | 2.13 | 2.11 | 1.86 | 2.04 | 1.81 | 2.02 | 1.67 | 1.76 | 1.78 | ..... |
| Fiber.....do.... | 2.26 | 2.15 | 2.28 | 2.35 | 2.72 | 2.79 | 2.78 | 2.80 | 2.65 | 3.01 | 2.63 | ..... |
| Pentosans.....do.... | 8.27 | 8.32 | 8.57 | 9.25 | 8.64 | 8.93 | 8.78 | 8.61 | 8.70 | 8.54 | 8.84 | ..... |
| Sugars.....do.... | 3.40 | 3.53 | 3.81 | 3.43 | 3.13 | 3.38 | 3.11 | 3.33 | 2.96 | 2.99 | 3.06 | ..... |
| Ash.....do.... | 1.87 | 1.84 | 1.82 | 2.05 | 1.99 | 1.97 | 2.29 | 1.97 | 2.09 | 2.07 | 2.22 | ..... |
| Phosphoric acid.....do.... | .84 | .79 | .86 | 1.02 | .85 | .81 | 1.08 | .86 | ..... | 1.09 | 1.21 | ..... |
| Potash.....do.... | .60 | .61 | .55 | .65 | .61 | .66 | .69 | .64 | ..... | .57 | .61 | ..... |
| Phosphoric acid in ash.....do.... | 45 | 43 | 47 | 50 | 43 | 41 | 47 | 41 | ..... | 53 | 50 | ..... |
| Potash in ash.....do.... | 32 | 33 | 30 | 28 | 31 | 31 | 30 | 30 | ..... | 28 | 27 | ..... |

[1] Owing to a severe drought the crop failed to mature.

TABLE I.—*Composition of wheat grown on different plats of soil in California, in Kansas, and in Maryland in 1909, 1910, 1911, and 1912*—Continued.

1911 crop.[1]

| Determination. | Wheat grown in California on— | | | | Wheat grown in Kansas on— | | | | Wheat grown in Maryland on— | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | California check soil. | California soil. | Kansas soil. | Maryland soil. | California soil. | Kansas soil. | Kansas check soil. | Maryland soil. | California soil. | Kansas soil. | Maryland soil. | Maryland check soil. |
| Physical properties: | | | | | | | | | | | | |
| Water.....per cent | ..... | ..... | ..... | ..... | 9.00 | 9.30 | 8.28 | 8.79 | 8.83 | 8.97 | 8.93 | 8.73 |
| Weight per 1,000 grains....grams | 38.4 | 34.6 | 37.7 | 23.5 | 12.9 | 13.3 | 12.5 | 13.8 | 27.4 | 29.4 | 27.1 | 26.4 |
| Weight per bushel.....pounds | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 60.5 | 62.2 | ..... | 59.9 |
| Flinty grains.....per cent | 89 | 51 | 46 | 100 | 100 | 98 | 97 | 98 | 25 | 20 | ..... | 30 |
| On water-free basis: | | | | | | | | | | | | |
| Nitrogen.....do | ..... | ..... | ..... | ..... | 3.70 | 4.09 | 4.07 | 3.97 | 2.00 | 2.20 | 2.37 | 2.31 |
| Protein (N×5.7).....do | 12.04 | 10.56 | 9.61 | 13.20 | 21.11 | 23.31 | 23.18 | 22.62 | 11.38 | 12.52 | 13.52 | 13.18 |
| Alcohol-soluble nitrogen.....do | .93 | .76 | .70 | .80 | ..... | ..... | ..... | ..... | .89 | .88 | 1.04 | .96 |
| Gliadin in protein.....do | 44 | 43 | 42 | 35 | ..... | ..... | ..... | ..... | 43 | 40 | 44 | 41 |
| Fat.....do | ..... | ..... | ..... | ..... | 1.94 | 1.95 | 1.83 | 2.12 | 2.04 | 2.05 | 1.83 | 1.87 |
| Fiber.....do | ..... | ..... | ..... | ..... | 2.95 | 2.94 | 3.17 | 3.21 | 2.41 | 2.33 | 2.49 | 2.44 |
| Pentosans.....do | ..... | ..... | ..... | ..... | 8.84 | 9.12 | 9.57 | 9.12 | 8.08 | 8.22 | 8.25 | 8.39 |
| Sugars.....do | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 3.25 | 3.45 | 3.33 | 3.34 |
| Ash.....do | 1.60 | 1.88 | 1.76 | 1.78 | 2.58 | 2.56 | 2.78 | 2.09 | 2.23 | 2.20 | 2.10 | 2.27 |
| Phosphoric acid.....do | ..... | ..... | ..... | ..... | 1.14 | 1.18 | ..... | .86 | 1.24 | 1.16 | 1.09 | 1.17 |
| Potash.....do | ..... | ..... | ..... | ..... | .69 | ..... | ..... | .64 | .67 | .65 | .65 | .67 |
| Phosphoric acid in ash.....do | ..... | ..... | ..... | ..... | 44 | 46 | ..... | 41 | 56 | 53 | 52 | 54 |
| Potash in ash.....do | ..... | ..... | ..... | ..... | 27 | ..... | ..... | 30 | 30 | 30 | 31 | 31 |

1912 crop.

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Physical properties: | | | | | | | | | | | | |
| Water.....per cent | 8.43 | 8.29 | 8.67 | 8.70 | 10.55 | 10.18 | 10.30 | 10.45 | 10.22 | 9.65 | 10.13 | 10.17 |
| Weight per 1,000 grains....grams | 29.3 | 30.5 | 31.8 | 23.9 | 22.0 | 21.4 | 28.6 | 16.2 | 25.7 | 25.7 | 19.19 | 22.4 |
| Weight per bushel.....pounds | ..... | 64.3 | 65.1 | ..... | ..... | 57.7 | ..... | ..... | 60.1 | 60.3 | ..... | ..... |
| Flinty grains.....per cent | 90 | 98 | 98 | 99 | 98 | ..... | ..... | ..... | 30 | 15 | ..... | 70 |
| On water-free basis: | | | | | | | | | | | | |
| Nitrogen.....do | 2.05 | 2.24 | 2.40 | 3.17 | 2.68 | 2.78 | 3.62 | 3.29 | 1.85 | 2.12 | 2.28 | 2.05 |
| Protein (N×5.7).....do | 11.68 | 12.77 | 13.68 | 18.07 | 15.29 | 15.87 | 20.65 | 18.78 | 10.54 | 12.11 | 13 | 11.68 |
| Alcohol-soluble nitrogen.....do | .85 | .97 | 1.02 | 1.40 | 1.14 | 1.20 | 1.64 | 1.35 | .70 | .85 | .89 | .83 |
| Gliadin in protein.....do | 41 | 43 | 43 | 44 | 42 | 43 | 45 | 41 | 38 | 40 | 39 | 40 |
| Fat.....do | 1.89 | 1.93 | 1.88 | 2.06 | 1.88 | 2.17 | 1.82 | 2.01 | 1.89 | 1.97 | 1.88 | 2 |
| Fiber.....do | 2.28 | 2.20 | 2.28 | 2.69 | 2.68 | 2.65 | 2.48 | 2.24 | 2.72 | 2.53 | 2.88 | 2.87 |
| Pentosans.....do | 8.08 | 7.95 | 8.05 | 8.70 | 8.31 | 8.27 | 8.52 | 8.98 | 8.44 | 8.62 | 9.36 | 9.03 |
| Sugars.....do | 3.56 | 3.78 | 3.80 | 3.91 | 3.37 | 3.41 | 3.12 | ..... | 2.95 | 3.08 | ..... | 3.32 |
| Ash.....do | 2.07 | 2.14 | 2.18 | 2.20 | 2.47 | 2.20 | 2.45 | 2.83 | 2.24 | 2.24 | 2.48 | 2.46 |
| Phosphoric acid.....do | 1.02 | 1.07 | 1.09 | 1.03 | 1.23 | 1.02 | 1.26 | 1.31 | 1.14 | 1.19 | 1.27 | 1.24 |
| Potash.....do | .62 | .62 | .62 | .59 | .74 | .67 | .66 | .79 | .71 | .72 | .80 | .79 |
| Phosphoric acid in ash.....do | 52 | 50 | 50 | 47 | 50 | 46 | 51 | 46 | 51 | 53 | 51 | 50 |
| Potash in ash.....do | 30 | 30 | 29 | 27 | 30 | 30 | 27 | 28 | 32 | 32 | 32 | 32 |

[1] The data for the 1911 samples grown in California were furnished by Prof. Shaw, of the University of California, under whose supervision the field work in that State was conducted.

TABLE II.—*Composition of wheat grown on plats of California, Kansas, and Maryland soils in California, in Kansas, and in Maryland.*

1909.

| Determination. | Analysis of wheat grown on— | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | California soil in— | | | Kansas soil in— | | | Maryland soil in— | | |
| | California. | Kansas. | Maryland. | California. | Kansas. | Maryland. | California. | Kansas. | Maryland. |
| Physical properties: | | | | | | | | | |
| Water.....per cent.. | 8.93 | ..... | 9.56 | 9.00 | ..... | 9.48 | 8.88 | ..... | 9.22 |
| Weight per 1,000 grains....grams.. | 34.6 | ..... | 21.2 | 36.4 | ..... | 23.0 | 25.4 | ..... | 22.2 |
| Weight per bushel.....pounds.. | 61.5 | ..... | ..... | 61.5 | ..... | ..... | ..... | ..... | ..... |
| Flinty grains.....per cent.. | 100 | ..... | 85 | 75 | ..... | 80 | 97 | ..... | ..... |
| On water-free basis: | | | | | | | | | |
| Nitrogen.....do.... | 2.78 | ..... | 2.69 | 2.01 | ..... | 2.57 | 2.03 | ..... | 2.34 |
| Protein (N × 5.7).....do.... | 15.84 | ..... | 15.33 | 11.46 | ..... | 14.65 | 11.57 | ..... | 13.34 |
| Alcohol-soluble nitrogen....do.... | 1.16 | ..... | 1.10 | .82 | ..... | 1.05 | .71 | ..... | .92 |
| Gliadin in protein.....do.... | 41 | ..... | 41 | 41 | ..... | 41 | 35 | ..... | 40 |
| Fat.....do.... | 1.82 | ..... | 2.16 | 1.82 | ..... | 2.05 | 1.84 | ..... | 2.15 |
| Fiber.....do.... | 2.33 | ..... | 2.69 | 2.43 | ..... | 2.62 | 2.39 | ..... | 2.59 |
| Pentosans.....do.... | 8.49 | ..... | 8.37 | 8.76 | ..... | 8.31 | 8.53 | ..... | 9.03 |
| Sugars.....do.... | 3.21 | ..... | 2.89 | 3.73 | ..... | 2.64 | 3.20 | ..... | 2.82 |
| Ash.....do.... | 1.63 | ..... | 2.39 | 1.63 | ..... | 2.30 | 1.90 | ..... | 2.09 |
| Phosphoric acid.....do.... | .68 | ..... | 1.23 | .70 | ..... | 1.18 | .89 | ..... | ..... |
| Potash.....do.... | .45 | ..... | ..... | .46 | ..... | .63 | .56 | ..... | ..... |
| Phosphoric acid in ash.....do.... | 42 | ..... | 51 | 43 | ..... | 51 | 47 | ..... | ..... |
| Potash in ash.....do.... | 28 | ..... | ..... | 29 | ..... | 27 | 30 | ..... | ..... |

1910.

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Physical properties: | | | | | | | | | |
| Water.....per cent.. | 9.00 | 9.39 | 9.00 | 9.67 | 9.03 | 10.66 | 8.99 | 9.12 | 9.13 |
| Weight per 1,000 grains....grams.. | 28.3 | 26.1 | 28.0 | 34.3 | 22.6 | 31.5 | 21.5 | 24.0 | 25.9 |
| Weight per bushel.....pounds.. | ..... | 58.3 | ..... | 61.8 | 56.9 | 57.7 | ..... | 55.8 | ..... |
| Flinty grains.....per cent.. | 100 | 99 | 0 | 70 | 100 | 0 | 100 | 100 | 0 |
| On water-free basis: | | | | | | | | | |
| Nitrogen.....do.... | 2.39 | 2.80 | 1.80 | 1.86 | 3.28 | 1.90 | 2.86 | 3.12 | 2.03 |
| Protein (N × 5.7).....do.... | 13.63 | 15.98 | 10.27 | 10.60 | 18.73 | 10.85 | 16.28 | 17.81 | 11.63 |
| Alcohol-soluble nitrogen.....do.... | 1.05 | 1.23 | ..... | .74 | 1.44 | .75 | ..... | 1.29 | ..... |
| Gliadin in protein.....do.... | 44 | 44 | ..... | 40 | 41 | 39 | ..... | 41 | ..... |
| Fat.....do.... | 2.13 | 1.86 | 1.67 | 2.13 | 2.04 | 1.76 | 2.11 | 2.03 | 1.38 |
| Fiber.....do.... | 2.15 | 2.72 | 2.65 | 2.28 | 2.79 | 3.01 | 2.35 | 2.89 | 2.65 |
| Pentosans.....do.... | 8.32 | 8.64 | 8.70 | 8.57 | 8.93 | 8.54 | 9.25 | 8.64 | 8.84 |
| Sugars.....do.... | 3.53 | 3.13 | 2.90 | 3.81 | 3.38 | 2.99 | 3.43 | 3.33 | 3.06 |
| Ash.....do.... | 1.84 | 1.99 | 2.09 | 1.82 | 1.97 | 2.07 | 2.05 | 1.97 | 2.22 |
| Phosphoric acid.....do.... | .79 | .85 | ..... | .86 | .81 | 1.09 | 1.02 | .80 | 1.22 |
| Potash.....do.... | .61 | .61 | ..... | .55 | .66 | .57 | .65 | .64 | .61 |
| Phosphoric acid in ash.....do.... | 43 | 43 | ..... | 47 | 41 | 53 | 50 | 41 | 59 |
| Potash in ash.....do.... | 33 | 31 | ..... | 30 | 31 | 28 | 28 | 30 | 27 |

1911.

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Physical properties: | | | | | | | | | |
| Water.....per cent.. | ..... | 9.00 | 8.83 | ..... | 9.30 | 8.97 | ..... | 8.59 | 8.93 |
| Weight per 1,000 grains....grams.. | 34.6 | 12.9 | 27.4 | 37.7 | 13.3 | 29.4 | 23.5 | 13.8 | 27.1 |
| Weight per bushel.....pounds.. | ..... | ..... | 60.5 | ..... | ..... | 62.2 | ..... | ..... | ..... |
| Flinty grains.....per cent.. | 51 | 100 | 25 | 46 | 93 | 20 | 100 | 98 | ..... |
| On water-free basis: | | | | | | | | | |
| Nitrogen.....do.... | ..... | 3.70 | 2.00 | ..... | 4.09 | 2.20 | ..... | 3.97 | 2.37 |
| Protein (N × 5.7).....do.... | 20.56 | 21.11 | 11.38 | 9.61 | 23.31 | 12.52 | 13.20 | 22.62 | 13.52 |
| Alcohol-soluble nitrogen.....do.... | .76 | ..... | .85 | .70 | ..... | .88 | .80 | ..... | 1.04 |
| Gliadin in protein.....do.... | 43 | ..... | 43 | 42 | ..... | 40 | 33 | ..... | 44 |
| Fat.....do.... | ..... | 1.94 | 2.04 | ..... | 1.95 | 2.03 | ..... | 2.12 | 1.83 |
| Fiber.....do.... | ..... | 2.95 | 2.41 | ..... | 2.94 | 2.33 | ..... | 3.21 | 2.49 |
| Pentosans.....do.... | ..... | 8.84 | 8.08 | ..... | 9.12 | 8.22 | ..... | 9.12 | 8.35 |
| Sugars.....do.... | ..... | ..... | 3.25 | ..... | ..... | 3.45 | ..... | ..... | 3.33 |
| Ash.....do.... | 1.88 | 2.58 | 2.23 | 1.76 | 2.56 | 2.20 | 1.78 | 2.89 | 2.10 |
| Phoshoric acid.....do.... | ..... | 1.14 | 1.24 | ..... | 1.18 | 1.16 | ..... | .86 | 1.09 |
| Potash.....do.... | ..... | .69 | .67 | ..... | ..... | .65 | ..... | .64 | .65 |
| Phosphoric acid in ash.....do.... | ..... | 44 | 56 | ..... | 46 | 53 | ..... | 41 | 52 |
| Potash in ash.....do.... | ..... | 27 | 30 | ..... | ..... | 30 | ..... | 30 | 32 |

TABLE II.—*Composition of wheat grown on plats of California, Kansas, and Maryland soils in California, in Kansas, and in Maryland*—Continued.

1912.

| Determination. | Analysis of wheat grown on— | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | California soil in— | | | Kansas soil in— | | | Maryland soil in— | | |
| | California. | Kansas. | Maryland. | California. | Kansas. | Maryland. | California. | Kansas. | Maryland. |
| Physical properties: | | | | | | | | | |
| Water.....per cent.. | 8.29 | 10.55 | 10.22 | 8.67 | 10.18 | 9.65 | 8.70 | 10.45 | 10.13 |
| Weight per 1,000 grains....grams.. | 30.5 | 22.0 | 25.7 | 31.8 | 21.4 | 25.7 | 23.9 | 16.2 | 19.9 |
| Weight per bushel.....pounds.. | 64.3 | ..... | 60.1 | 65.1 | 57.7 | 60.3 | ..... | ..... | ..... |
| Flinty grains.....per cent.. | 98 | 98 | 30 | 98 | 100 | 75 | 99 | ..... | ..... |
| On water-free basis: | | | | | | | | | |
| Nitrogen.....do.... | 2.24 | 2.68 | 1.85 | 2.40 | 2.78 | 2.12 | 3.17 | 3.29 | 2.28 |
| Protein (N × 5.7).....do.... | 12.77 | 15.29 | 10.54 | 13.68 | 15.87 | 12.11 | 18.07 | 18.78 | 13 |
| Alcohol-soluble nitrogen.....do.... | .97 | 1.14 | .70 | 1.02 | 1.20 | .85 | 1.40 | 1.35 | 89 |
| Gliadin in protein.....do.... | 43 | 42 | 38 | 43 | 43 | 40 | 44 | 41 | 39 |
| Fat.....do.... | 1.93 | 1.88 | 1.89 | 1.88 | 2.17 | 1.97 | 2.05 | 2.01 | 1.88 |
| Fiber.....do.... | 2.20 | 2.68 | 2.72 | 2.28 | 2.65 | 2.53 | 2.69 | 3.24 | 2.88 |
| Pentosans.....do.... | 7.95 | 8.31 | 8.44 | 8.05 | 8.27 | 8.62 | 8.70 | 8.98 | 9.36 |
| Sugars.....do.... | 3.58 | 3.37 | 2.95 | 3.80 | 3.41 | 3.08 | 3.91 | ..... | ..... |
| Ash.....do.... | 2.14 | 2.47 | 2.24 | 2.18 | 2.20 | 2.24 | 2.20 | 2.83 | 2.48 |
| Phosphoric acid.....do.... | 1.07 | 1.23 | 1.14 | 1.09 | 1.02 | 1.19 | 1.03 | 1.31 | 1.27 |
| Potash.....do.... | .63 | .74 | .71 | .62 | .67 | .72 | .59 | .79 | .80 |
| Phosphoric acid in ash.....do.... | 50 | 50 | 51 | 50 | 46 | 53 | 47 | 46 | 51 |
| Potash in ash.....do.... | 30 | 30 | 32 | 29 | 30 | 32 | 27 | 28 | 32 |

TABLE III.—*Averages and extremes in wheat grown on plats of California, Kansas, and Maryland soils in California, in Kansas, and in Maryland.*[1]

| Determination. | California. | | | | Kansas. | | | | Maryland. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Averages. | | Extremes. | | Averages. | | Extremes. | | Averages. | | Extremes. | |
| | Mean. | Divergence from mean. | Minimum. | Maximum. | Mean. | Divergence from mean. | Minimum. | Maximum. | Mean. | Divergence from mean. | Minimum. | Maximum. |
| Physical properties: | | | | | | | | | | | | |
| Water.....per cent.. | 8.98 | 0.31 | 8.29 | 9.68 | 9.53 | 0.57 | 8.79 | 10.55 | 9.53 | 0.46 | 8.83 | 10.66 |
| Weight per 1,000 grains, grams..... | 30.2 | 4.5 | 21.5 | 37.7 | 19.1 | 4.5 | 12.9 | 26.1 | 25.0 | 2.7 | 19.9 | 31.5 |
| Weight per bushel, pounds..... | 62.8 | 1.5 | 61.5 | 65.1 | 57.2 | .8 | 55.8 | 58.3 | 60.1 | 1 | 57.7 | 62.2 |
| Flinty grains, per cent.. | 86 | 17 | 46 | 100 | 99 | 1 | 98 | 100 | 35 | 30 | 0 | 85 |
| On water-free basis: | | | | | | | | | | | | |
| Nitrogen.....per cent.. | 2.42 | .35 | 1.86 | 3.17 | 3.30 | .41 | 2.68 | 4.09 | 2.18 | .23 | 1.80 | 2.69 |
| Protein (N × 5.7), per cent..... | 13.11 | 2.01 | 9.61 | 18.07 | 18.83 | 2.34 | 15.29 | 23.31 | 12.43 | 1.29 | 10.27 | 15.33 |
| Alcohol-soluble nitrogen.....per cent.. | .92 | .18 | .70 | 1.40 | 1.27 | .09 | 1.14 | 1.44 | .90 | .10 | .70 | 1.10 |
| Gliadin in protein, per cent..... | 41 | 2 | 35 | 44 | 42 | 1 | 41 | 44 | 40 | 1 | 38 | 44 |
| Fat.....per cent.. | 1.97 | .12 | 1.82 | 2.13 | 2 | .08 | 1.86 | 2.17 | 1.94 | .14 | 1.67 | 2.16 |
| Fiber.....do.... | 2.34 | .11 | 2.15 | 2.69 | 2.89 | .18 | 2.65 | 3.21 | 2.63 | .13 | 2.33 | 3.01 |
| Pentosans.....do.... | 8.43 | .22 | 7.95 | 9.25 | 8.76 | .26 | 8.27 | 9.12 | 8.56 | .29 | 8.08 | 9.36 |
| Sugars.....do.... | 3.61 | .22 | 3.21 | 3.97 | 3.32 | .08 | 3.13 | 3.41 | 3.03 | .18 | 2.64 | 3.45 |
| Ash.....do.... | 1.90 | .16 | 1.63 | 2.20 | 2.30 | .28 | 1.97 | 2.83 | 2.22 | .09 | 2.07 | 2.48 |
| Phosphoric acid.....do.... | .90 | .13 | .68 | 1.09 | 1.02 | .17 | .80 | 1.31 | 1.18 | .05 | 1.09 | 1.27 |
| Potash.....do.... | .57 | .06 | .45 | .65 | .68 | .04 | .61 | .79 | .67 | .05 | .57 | .80 |
| Phosphoric acid in ash, per cent..... | 47 | 2 | 42 | 50 | 45 | 4 | 41 | 50 | 53 | 2 | 51 | 59 |
| Potash in ash, per cent.. | 29 | 1 | 27 | 33 | 30 | 1 | 27 | 31 | 30 | 2 | 27 | 32 |

[1] Not including check plats.

TABLE IV.—*Averages and extremes in wheat grown in California, in Kansas, and in Maryland on plats of California, Kansas, and Maryland soils.*[1]

| Determination. | California soil. | | | | Kansas soil. | | | | Maryland soil. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Averages. | | Extremes. | | Averages. | | Extremes. | | Averages. | | Extremes. | |
| | Mean. | Divergence from mean. | Minimum. | Maximum. | Mean. | Divergence from mean. | Minimum. | Maximum. | Mean. | Divergence from mean. | Minimum. | Maximum. |
| Physical properties: | | | | | | | | | | | | |
| Water.....per cent.. | 9.35 | 0.53 | 8.29 | 10.22 | 9.46 | 0.47 | 8.67 | 10.66 | 9.29 | 0.48 | 8.70 | 10.45 |
| Weight per 1,000 grains, grams... | 26.5 | 4.5 | 12.9 | 34.6 | 27.9 | 6.1 | 13.3 | 37.7 | 22.1 | 3.1 | 13.8 | 27.1 |
| Weight per bushel, pounds | 60.9 | 1.5 | 58.3 | 64.3 | 60.4 | 2.2 | 56.9 | 65.1 | (2) | (2) | (2) | (2) |
| Flinty grains, per cent.. | 71 | 33 | 0 | 100 | 69 | 26 | 0 | 100 | 85 | 24 | 0 | 100 |
| On water-free basis: | | | | | | | | | | | | |
| Nitrogen.....per cent.. | 2.48 | .44 | 1.80 | 3.70 | 2.52 | .53 | 1.86 | 4.09 | 2.75 | .53 | 2.03 | 3.97 |
| Protein (N × 5.7), per cent..... | 13.88 | 2.56 | 10.27 | 21.11 | 13.94 | 3.05 | 9.61 | 23.32 | 15.44 | 2.97 | 11.57 | 22.62 |
| Alcohol-soluble nitrogen.....per cent.. | 1 | .16 | .70 | 1.23 | .94 | .18 | .70 | 1.44 | 1.05 | .22 | .72 | 1.40 |
| Gliadin in protein, per cent..... | 42 | 1 | 38 | 44 | 41 | 1 | 39 | 43 | 40 | 3 | 35 | 44 |
| Fat.....per cent.. | 1.93 | .11 | 1.67 | 2.16 | 1.98 | .11 | 1.76 | 2.17 | 1.97 | .12 | 1.78 | 2.15 |
| Fiber.....do.... | 2.55 | .22 | 2.15 | 2.95 | 2.59 | .22 | 2.28 | 3.01 | 2.73 | .24 | 2.35 | 3.24 |
| Pentosans.....do.... | 8.41 | .21 | 7.95 | 8.84 | 8.48 | .28 | 8.05 | 9.12 | 8.87 | .28 | 8.25 | 9.36 |
| Sugars.....do.... | 3.33 | .28 | 2.89 | 3.90 | 3.48 | .24 | 2.99 | 3.81 | 3.39 | .22 | 2.82 | 3.91 |
| Ash.....do.... | 2.13 | .23 | 1.93 | 2.58 | 2.08 | .11 | 1.63 | 2.50 | 2.16 | .20 | 1.78 | 2.83 |
| Phosphoric acid..do.... | 1.04 | .18 | .68 | 1.24 | 1.03 | .15 | .70 | 1.19 | 1.05 | .15 | .80 | 1.31 |
| Potash.....do.... | .64 | .06 | .45 | .74 | .61 | .07 | .46 | .72 | .66 | .06 | .56 | .86 |
| Phosphoric acid in ash, per cent..... | 48 | 4 | 42 | 56 | 48 | 4 | 41 | 53 | 48 | 4 | 41 | 59 |
| Potash in ash, per cent.. | 30 | 1 | 27 | 33 | 29 | 1 | 27 | 32 | 29 | 2 | 27 | 32 |

[1] Not including check plats. [2] Only 1 sample.

TABLE V.—*Averages and extremes for the years 1909, 1910, 1911, and 1912 in wheat on disturbed and undisturbed plats*[1] *for all localities (California, Kansas, and Maryland) and years.*

| Determination. | Disturbed. | | | | Undisturbed. | | | |
|---|---|---|---|---|---|---|---|---|
| | Averages. | | Extremes. | | Averages. | | Extremes. | |
| | Mean. | Divergence from mean. | Minimum. | Maximum. | Mean. | Divergence from mean. | Minimum. | Maximum. |
| Physical properties: | | | | | | | | |
| Water.....per cent.. | 9.31 | 0.51 | 8.29 | 10.18 | 9.33 | 0.65 | 8.28 | 10.30 |
| Weight per 1,000 grains..grams.. | 25.8 | 4.9 | 13.3 | 34.6 | 27.6 | 5.7 | 12.5 | 38.4 |
| Weight per bushel.....pounds.. | Only two samples. | | | | | | | |
| Flinty grains.....per cent.. | 92 | 12 | 51 | 100 | 96 | 4 | 89 | 100 |
| On water-free basis: | | | | | | | | |
| Nitrogen.....per cent.. | 2.78 | .45 | 2.24 | 4.09 | 2.76 | .66 | 2.05 | 4.07 |
| Protein (N × 5.7).....do.... | 15.25 | 2.84 | 10.56 | 23.31 | 15.32 | 3.62 | 11.68 | 23.18 |
| Alcoholic-soluble nitrogen, per cent..... | 1.06 | .15 | .76 | 1.44 | 1.09 | .23 | .83 | 1.64 |
| Gliadin in protein.....per cent.. | 42 | 1 | 39 | 44 | 43 | 2 | 40 | 46 |
| Fat.....do.... | 1.97 | .11 | 1.82 | 2.17 | 1.86 | .08 | 1.67 | 2.01 |
| Fiber.....do.... | 2.55 | .26 | 2.15 | 2.94 | 2.56 | .29 | 2.18 | 3.17 |
| Pentosans.....do.... | 8.59 | .41 | 7.95 | 9.36 | 8.61 | .35 | 8.08 | 9.59 |
| Sugars.....do.... | 3.44 | .14 | 3.21 | 3.78 | 3.34 | .14 | 3.11 | 3.56 |
| Ash.....do.... | 2.09 | .23 | 1.63 | 2.56 | 2.16 | .30 | 1.69 | 2.78 |
| Phosphoric acid.....do.... | .96 | .17 | .68 | 1.27 | 1.06 | .15 | .79 | 1.26 |
| Potash.....do.... | .64 | .06 | .45 | .80 | .64 | .07 | .48 | .79 |
| Phosphoric acid in ash.....do.... | 46 | 4 | 41 | 52 | 49 | 3 | 45 | 54 |
| Potash in ash.....do.... | 31 | 1 | 28 | 33 | 30 | 1 | 27 | 32 |

[1] Only data that are strictly comparable are used. Disturbed-plat data are used only if the determinations for the corresponding check plats were also made, and vice versa.

## PHYSICAL CHARACTERISTICS

### WEIGHT OF 1,000 GRAINS OF WHEAT

In California the grains were almost uniformly plump and heavy, not varying far from 30 grams for each thousand, except in the case of the samples grown on the soil obtained from Maryland. In Kansas they were less plump, 1,000 grains weighing about 23 grams in 1910, 13 grams in 1911, and 20 grams in 1912. In Maryland the weight of 1,000 grains was quite uniform throughout the series of four years. As a rule, the size of the grains in each locality for each year was uniform, irrespective of the type of soil in which they grew. There were, however, a few notable exceptions to this rule: The grain grown on Maryland soil in each year from 1909 to 1912 in California, as well as that grown on the Maryland soil in 1912 in Kansas, was decidedly lighter in weight than that grown in the same locality on the other soils. This would seem to indicate that some soils play an important part in influencing the size of the grain.

Between the localities there was usually a much greater difference in the weight of 1,000 grains than was noted between the soils. (See Table II.) The weight of 1,000 grains, then, is distinctly dependent, as a rule, on climatic or seasonal conditions rather than on soil characteristics. The fact that environment plays the chief rôle in influencing the weight is again brought out in the tables of averages, which show a great difference, for example, 30.2, 19.1, and 25.6 grams for California, Kansas, and Maryland, respectively, when averaged by localities (see Table III), and a relative uniformity of 26.5, 27.9, and 22.1 grams, respectively, when averaged by source of soil (see Table IV).

Table I shows that in about 80 per cent of the samples investigated the weight of 1,000 grains of seed grown on different soils in any one locality was sufficiently uniform to permit the conclusion that climate and not soil is the chief factor affecting the size of the grain. From Table III it is seen that the California-grown samples averaged the heaviest and the Kansas-grown samples the lightest.

### WEIGHT OF ONE BUSHEL OF WHEAT

The weight of a bushel of wheat runs more or less parallel with the weight of 1,000 grains. If the samples weighing over 61 pounds to the bushel are compared with those weighing less than 60 pounds, it will be found that the weight of 1,000 grains of the former was, on an average, 33.4 grams, and that of the latter, 25 grams. In many cases, owing to the small amount of material, it was impossible to make a weight-by-bushel determination.

### FLINTY GRAINS

Classifying the grains of each sample into those which were wholly dark or flinty and those which appeared to be light brown or mealy, a remarkable uniformity is found in the groups arranged by locality in

which they grew (see Table I) and a dissimilarity in groups arranged by the source of soil (see Table II). The averages by localities (see Table III) differ greatly, being 86, 99, and 35 per cent for California, Kansas, and Maryland, respectively. The averages by soils are very uniform, being 71, 69, and 85 per cent, respectively. (See Table IV.)

These averages do not show the great variations actually found in the different regions in any one year, for seasonal variations of the individual localities tend to equalize the averages. In Table I, for example, while the samples grown in California and Kansas in 1910 in each of the three soils were for the most part flinty, those grown in the three soils in Maryland were all more or less starchy or mealy. Similar figures are noted in 1911, when the Kansas samples grown on all three soils yielded wheat which was practically 100 per cent flinty, while on the same soils in Maryland the percentage of flinty kernels was less than half as great.

## CHEMICAL CONSTITUENTS

In considering the composition of the wheat it will be seen that many of the organic and inorganic constituents undergo as great variations as have already been noted with respect to the physical characteristics. On the other hand, there are a number of these constituents which showed very little variation, or no regularity in such variations as exist. Among those showing but little variation may be mentioned the gliadin number and the potash in the ash, and among those showing no pronounced regularity in the variations are the fat, fiber, pentosans, and sugars. With those exhibiting variations of a regular character belong particularly the nitrogen and protein, the ash, the phosphoric acid, and the phosphoric acid in the ash.

### PROTEIN

As the protein of wheat is its most important constituent, it will be of more than usual interest to note the changes produced by difference of soil and by change of environment. As a rule, there was a remarkable uniformity each year among the samples grown in any one locality, independent of the soil upon which they grew. Thus, in 1910, 1911, and 1912 the protein in wheat grown in California was almost uniformly low, about 13 per cent; in Maryland it was also low, about 11 per cent; while in Kansas it was high, nearly 18 per cent. This fact is more clearly brought out in Table III, which shows the average protein content to be 13.11, 18.83, and 12.43 per cent for California, Kansas, and Maryland, respectively.

In Table IV, where the results are arranged according to source of soil, it will be seen that the wheats grown on California soil in all three localities had an average protein content of 13.88 per cent, those grown on Kansas soil, 13.94 per cent, and those on Maryland soil, 15.44 per cent. This shows a rather striking uniformity and again emphasizes the rela-

tively small rôle played by the soil in influencing the protein content of wheat. There was a greater similarity between the protein contents of the samples grown in Maryland and California, both relatively humid regions, than between the protein contents of samples from either of these localities and those from Kansas, which has a comparatively dry climate.

There are a few exceptions, however, to the rule that soil influences the composition of wheat to only a slight degree. Among the most striking of these were the protein results obtained in 1909 in California on California soil, in 1910 and 1912 in California on Maryland soil, as well as in Kansas on Kansas check soil; that is, 4 out of 42 cases did not follow the general rule. Since about 90 per cent of the results obtained followed the general rule, and the exceptions noted were in different localities and on different soils and not always on the same soil in any locality, it is probably safe to assume that the contrary results given by the other 10 per cent of samples were accidental. These few exceptions among the prevailing regularities may serve to emphasize the fact, too frequently overlooked in plat experiments of this kind where many factors may affect the results, that a regularity needs to be traced through a great number of individual instances before it is safe to draw conclusions from it. Thus, in this experiment a consideration of the data from the 1909 crop alone might show that the soil has a marked determining influence upon the protein content and that the California soil tends to produce a wheat of relatively high protein content. That such a conclusion would be erroneous is evidenced by practically all the data of the three following years, for in no other case during 1910, 1911, and 1912 was there a larger amount of protein in wheat grown on the California soil than in that grown on the two other soils. In fact, those wheats were invariably lower in protein content.

While these exceptions may be considered as purely accidental, the following question is suggested by such variations from the rule: Is there in the physical, chemical, or biological characteristics of the soil a real difference which at first exerts a determining influence on the composition of the crop, but which may be obliterated in the course of a year or two after putting the soil down in a different locality? Some weight is lent to such a hypothesis by the fact that the slight differences in protein content in the crops grown in Maryland the first year after the exchange of soils were much the same as the exceptionally great differences in the crops grown in California. Unfortunately, the Kansas crop was a complete failure, and it is impossible, therefore, to know in what way the soil there would have influenced the composition of the crop during the first year. To answer this question, more observations during the first few years of similar soil exchange experiments would be necessary, using larger plats to partly eliminate any tendency for soils to equalize after being together in one locality, if such a tendency does exist.

It seems justifiable to conclude that climate is the principal factor influencing the protein content of wheat, and that soils, when used as in this experiment, have little or no influence.

### GLIADIN IN PROTEIN

With very few exceptions, the amount of alcohol-soluble nitrogen or gliadin bore a close relation to that of total nitrogen. The percentage of gliadin in the wheat grown on the different soils in the three localities during the years 1909 to 1912 remained practically constant at 41 per cent, except in the case of wheat grown on Maryland soil and on California check soil in California in 1909, and on Maryland soil in California in 1911. These 3 exceptions out of 42 samples can not be explained and must be assumed to be accidental. From Table II it would seem that those samples grown on Maryland soil in California in 1909, 1911, and 1912 and in Maryland in 1912 formed exceptions to the rule. When general averages are considered, however, practically no differences in gliadin number due either to difference of soils or to change of seasonal conditions are noted. Table III gives the average gliadin numbers of the samples grown on each of the three soils in California as 41; in Kansas, 42; and in Maryland, 40. Table IV shows the gliadin number of the wheats grown on California soil in each of the three localities to be 42; on Kansas soil, 41; and on Maryland soil, 40. There seems to be a slight tendency for the Maryland soil to be low in gliadin. The differences are, however, small and probably no weight should be given them.

### FAT

The amounts of fat agreed very closely in the case of wheat grown on the different soils in any one locality, only 3 out of 42 samples showing a greater variation than 0.2 per cent, which may be assumed to be the limit of error for fat determinations. When averaged by locality, the results were 1.97, 2.00, and 1.94 per cent for wheat grown in California, Kansas, and Maryland, respectively. When averaged by source of soils, the results were 1.93, 1.98, and 1.97 per cent for samples grown on California, Kansas, and Maryland soils, respectively. The results taken as a whole indicate that fat is not affected to any great extent by climatic or soil conditions.

### FIBER

The fiber showed a somewhat greater variation in amount than did the fat. The results as a whole indicate that a greater influence is exerted by seasonal or climatic changes than by differences in soils. This is shown in Table III, with the average fiber content of 2.34, 2.89, and 2.63 per cent in the wheats grown on the three soils in California, Kansas, and Maryland, respectively.

The wheat grown in the three localities on California soil gave 2.55 per cent of fiber, on Kansas soil, 2.59, and on Maryland soil, 2.73. (See

Table IV.) These averages agree with one another more closely than do those in Table III, proving that soils play a minor rôle in influencing the fiber content.

PENTOSANS

The pentosan content followed generally the fiber content, being high where the fiber content was high and low where the fiber content was low.

SUGARS

The sugar content of the samples grown in California was somewhat higher than that of those grown in Kansas or in Maryland.

ASH

If soil itself has any influence on the composition of the wheat, it is reasonable to expect that the mineral constituents especially will be thus influenced. Even here, however, in the case of ash, the soil factor is a minor or negligible one. There was a decided regularity in the ash content, and, like the physical properties and the protein content, this regularity consisted in an approximately uniform ash content of the samples grown during any one year in any one locality. Thus, during each of the four years California produced from all soils crops with a low ash content of about 1.9 per cent, while Kansas produced crops relatively higher in ash, averaging 2.30 per cent, and Maryland nearly as high, varying somewhat, however, from year to year, with an average of 2.22 per cent. The average ash content of all crops grown on each of the three soils, irrespective of the locality, showed but slight variation, being 2.13, 2.08, and 2.16 per cent for California, Kansas, and Maryland soils, respectively.

PHOSPHORIC-ACID CONTENT OF THE WHOLE WHEAT AND OF THE ASH

In most cases the amount of phosphoric acid rose or fell in the same proportion as the ash, so that the percentage of phosphoric acid in the ash remained practically constant, averaging 47 per cent for California, 45 per cent for Kansas, and varying from 41 to 51 per cent in these two localities. The crops grown in Maryland, however, on all soils had a strangely high amount of phosphoric acid, averaging 53 per cent of the ash and varying from 51 to 59 per cent. There is no explanation for the fact that in Maryland all the soils used in this experiment supplied to the grain mineral constituents with a percentage of phosphoric acid much higher than that supplied by the same soils in California and in Kansas. It was apparently due to some climatic or seasonal conditions prevailing in Maryland. The kind of soil did not, however, affect the amount of phosphoric acid in the wheat or in the ash, for Table IV shows that the average in the wheat grown in the three localities on plats of California soil was 1.04 per cent, on plats of Kansas soil, 1.03 per cent, and on plats of Maryland soil, 1.05 per cent, and the phosphoric acid in the ash was 48 per cent in each case.

POTASH CONTENT OF THE WHOLE WHEAT AND OF THE ASH

The potash in the wheat, like the total ash, was seemingly influenced more by climatic and seasonal variations than by the soil, so that the amount of potash in all samples rose or fell in practically the same proportion as the amount of total ash, and the percentage of potash in the ash—about 30 per cent—remained very nearly constant for all localities, soils, and seasons included in the experiment. This is further shown by the similarity of the averages, whether by locality (see Table III), with averages of 29, 30, and 30 per cent for California, Kansas, and Maryland, respectively, or by soils (see Table IV) with averages of 30, 29, and 29 per cent, respectively.

## CORRELATION BETWEEN PHYSICAL PROPERTIES AND CHEMICAL CONSTITUENTS

Although the relationship or interdependence between the physical properties and chemical constituents does not show in these results as markedly as might be expected, such relationships may be distinctly traced in some of the constituents. Thus, as has often been pointed out by others, a distinct correlation exists between the protein content and the physical appearance or between the protein content and the weight of 1,000 grains, high protein being more or less parallel with flintiness and with lightness of grains. The table of averages (see Table III) shows that the Kansas samples, containing 18.83 per cent of protein, averaged 99 per cent of flinty grains and weighed at the rate of 19.1 grams for 1,000 grains, while the Maryland samples, containing 12.43 per cent of protein, averaged but 35 per cent of flinty kernels and weighed 25.6 grams for 1,000 grains, and the California samples, containing 13.11 per cent of protein, averaged 86 per cent of flinty grains and weighed as high as 30.2 grams for 1,000 grains. The results in Table IV show a similar tendency in these respects, the samples grown on Maryland soils in the three localities being somewhat richer in protein and having at the same time more flinty kernels and weighing less for each 1,000 grains than the samples grown on California or Kansas soils. The differences in this case, however, were very much less notable than those due to climatic variations. (See Table III.) There was a less noticeable parallelism between the fiber and pentosans, a high fiber content, as a rule, being accompanied by a high pentosan content, and vice versa. The California-grown samples, which were the heaviest, contained the smallest amount of fiber and pentosans, while the Kansas samples, which were the lightest, contained the greatest amount.

The fact that the ash and protein contents were low in the California-grown samples and high in the Kansas-grown samples might lead one to expect that the ash was a function of the protein content. This is not borne out by an examination of Table III, where it is seen that the ash

of the samples grown in Maryland was appreciably higher than that of the samples grown in California, while the protein of the former was less than that of the latter. On the other hand, the ash content of the Kansas samples was only slightly higher than that of the Maryland-grown samples, although the protein content of the former was 50 per cent higher than that of the latter.

### COMPARISON BETWEEN RESULTS FROM DISTURBED AND UNDISTURBED PLATS OF THE SAME SOIL

Attention has thus far been directed primarily to the composition of the wheat samples grown for several years in each locality on each of the three soil plats which had been taken up in 3-inch layers and interchanged among the three localities. As previously mentioned, a check plat of equal size, in which the soil had not been disturbed, was planted each year in each locality, and samples from it were analyzed for comparison. A fear that manipulation of the soil would produce abnormal conditions, influencing the character of the crop, was not justified by these results (Table V), at least not as evidenced by the physical appearance and the chemical composition. The slight differences between the crops from the disturbed and undisturbed plats of the same soil are apparently either accidental or due to errors in sampling or in analysis. This is further borne out by the results from both the seed-exchange experiments [1] and from the soil-exchange experiments (pp. 278–281). It is simply a verification of the conclusion already drawn, that the soil factor plays but a very subordinate part or is entirely devoid of influence in determining these characteristics in the crop.

Such great differences exist in respect to one constituent, however, that they must be classed as exceptions to the rule. The percentage of phosphoric acid averaged 0.96 per cent in the samples from disturbed plats and 1.06 per cent in those from undisturbed plats, or, if expressed as the percentage of phosphoric acid in the ash, it is 46 and 49 per cent, respectively. It might seem that the undisturbed soil could give a little more phosphoric acid to the grain than the disturbed soil. These differences, being only slightly greater than the limit of error in analytical work, probably have no significance.

### CONCLUSIONS

As is to be expected in plat work in the field, especially with such small plats as were used for these experiments, there are many variations in the results which seem accidental, in that they can not be interpreted according to any definite law. There are, however, certain variations which appear with such regularity that important conclusions may be drawn from them.

An inspection of the tables should show whether climatic conditions or soil characteristics have a strong determining influence upon the

[1] Le Clerc and Leavitt. Op. cit.

properties or composition of the crop. If the adjacent data in Table I under each locality are similar and distinctly unlike the corresponding group in another region, it is evident that the locality—that is, the climate—has exerted a strong influence. Likewise, if a similarity exists in the data in the adjacent columns in Table II as regards crops from the same soil and there is a distinct difference between them and the corresponding data from other soils, it is clear that the soils in themselves have a determining influence, regardless of the locality in which the soils happen to be.

To avoid erroneous conclusions concerning any property or constituent, due to accidental differences occurring in individual groups of data, it is necessary to make a survey of all the data on hand regarding that property or constituent. In a measure the averages drawn from the several groups of data furnish quantitative values which may indicate the persistence or the nonpersistence of such differences. The average divergences from these means, together with the minima and the maxima, supply further quantitative evidence along this line. Such averages and the corresponding minima and maxima are brought together in Tables III, IV, and V.

This experiment, covering a period of four years, in which three fairly good wheat soils, one each from California, Kansas, and Maryland, were put down side by side in each of these three localities and cropped with the same variety of wheat, shows that the soil does not exert the chief or preponderating influence in determining the physical properties or the chemical constituents of the grain crop. No attempt has been made to trace out from these experiments the manner in which the climatic factors thus exert the chief determining influence on the composition of the wheat crop. The following possibilities may, however, be considered:

(1) Differences in humidity may cause a difference in the transpiration of the plants, which in turn may react on the composition of the crop.

(2) Variations in the amount and distribution of sunlight may influence diversely the photosynthesis of the plants.

(3) Differences in temperature and in the succession of hot and cold periods may cause varying vegetative activities in the plants.

(4) The climatic differences, such as the humidity, rainfall, temperature, and sunlight, may bring about changes in the physical, chemical, or biological characteristics of the soil which in turn may react on the crop.

From this it should not be assumed that it is impossible for soil which has been transferred from one locality to another to become so changed by climatic environment that the character of the wheat grown thereon would be approximately the same as that grown in soil belonging to the second locality. This has been suggested to explain the facts observed during this experiment—namely, that wheats grown on the three soils in Kansas are very different from the same variety of wheat grown

on the same soils transported to Maryland. In view of the further fact, generally accepted by agriculturists, that the same variety of wheat grown over certain large areas having similar climatic conditions possesses approximately the same physical and chemical characteristics, notwithstanding the inherent differences in soil on which they were grown or the differences of fertilizers applied to these soils, it would seem that climate plays a greater rôle than soils as such in influencing the composition of wheat.

Of the biological factors, those bearing on nitrification might be the most influential in affecting the protein content of the crop. Yet it is a noteworthy fact that the application of nitrate as a fertilizer increases the protein content of the crop to only a slight degree. Considering the great difference existing between the protein of the Maryland and Kansas crops, it may therefore be concluded that even if nitrification were greater in Maryland soil transferred to Kansas than in Maryland soil in Maryland, that fact would not be sufficient to explain the wide variation between the composition of the wheat grown on the four plats in Maryland and on the four plats in Kansas.

It is also shown that the crops from the plats which had been taken up in 3-inch layers and replaced had approximately the same physical and chemical characteristics throughout as the crops from the corresponding plats which had not been thus disturbed. On the other hand, it is shown that the climatic factors collectively have a strong determining influence, especially upon the crude-protein content, the ash content, and the percentage of phosphoric acid in the ash. The results from this experiment thus harmonize with the findings previously published[1]—namely, that environment rather than what has been usually termed heredity is the major factor in determining the physical and chemical characteristics of the wheat crop. They indicate, further, that it is the climatic environment which exercises the primary influence of the environmental factors.

[1] Le Clerc and Leavitt. Op. cit.

# A DROUGHT-RESISTING ADAPTATION IN SEEDLINGS OF HOPI MAIZE

By G. N. Collins,

*Botanist, Office of Acclimatization and Adaptation of Crop Plants, Bureau of Plant Industry*

## INTRODUCTION

A study of the maize grown by the Hopi, Zuni, and Navajo Indians of New Mexico and Arizona has brought to light an adaptive character that promises to be of economic importance in dry regions where germination is uncertain.

These southwestern Indians have preserved from pre-Columbian times a type of maize able to produce fair crops in regions where the better known varieties of the East fail for lack of sufficient water. An important factor in the drought resistance of this type of corn is its ability to force the growing shoot of the seedling to the surface of the soil when planted at a depth of a foot or more. At such depths less specialized varieties die before reaching the surface.

The literature of corn contains reports of many experiments conducted to determine the proper depth of planting, but the results are confusing and contradictory. It has generally been realized that the optimum depth is influenced by differences in soil and climate, but that the proper depth might vary with different varieties seems not to have been appreciated. The experiments referred to later, as well as many unpublished data showing the varying behavior of types when planted at different depths, indicate that it is unsafe and unscientific to generalize with respect to cultural factors without taking type, varietal, and even individual differences into account.

## MORPHOLOGY OF THE MAIZE SEEDLING

To explain this drought-resistant character, it will be necessary to discuss briefly the different parts of a maize seedling. (See fig. 1.) The primary root, or radicle, which is the first organ to emerge from the germinating seed, is soon followed by the shoot or plumule. Inclosing the shoot is the cotyledonary sheath, or coleoptyle, a tubular organ which is closed and pointed at the upper end. Between the base of the coleoptyle and the seed the axis is somewhat elongated. With seeds germinated in the laboratory this elongation is so slight that it might easily be overlooked. Nevertheless, this small organ has not escaped the notice of morphologists, and its nature has been the subject of much discussion. It has

been variously called "hypocotyl," "mesocotyl," and "epicotyl." By some it is held to be an internode, by others merely an elongated node.

The choice of a name for the organ depends on the interpretation of the homologies of the other parts of the embryo, particularly as to what is considered as constituting the cotyledon. If the sheath, or coleoptyle, be thought of as the cotyledon, the most appropriate name would be hypocotyl. Although this interpretation was accepted by Richard (1811),[1] Hofmeister (1858), and Sachs (1875), there seems to be little evidence in its favor and it is summarily dismissed by other morphologists.

The two remaining views are as follows:

(1) The scutellum alone is the cotyledon, the epiblast (absent in maize) representing a second leaf and the coleoptyle a third. The elongated axis between the coleoptyle and scutellum is thus considered an internode and is then given the name "epicotyl." Among the supporters of this hypothesis are the following: Warming (1879–80), Hackel (1887), Bruns (1892), Van Tieghem (1897), and Holm (1908–9).

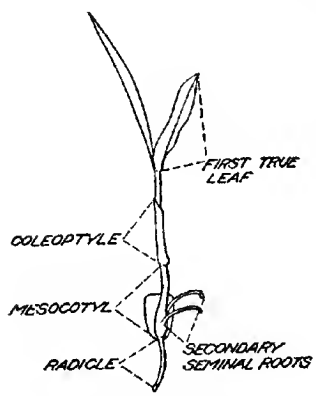



FIG. 1.—Diagram of seedling maize plant, giving terminology of parts.

(2) All these organs, scutellum, epiblast, and coleoptyle, are viewed as parts of a more highly specialized cotyledon, in which case the term "mesocotyl" is applied to the portion between the coleoptyle and scutellum. With various modifications this last interpretation is adopted by Van Tieghem (1872), Hagelmaier (1874), Klebs (1881), Schlickum (1896), Čelakovský (1897), and Goebel (1905).

Van Tieghem originally subscribed to the view that the coleoptyle was a part of the cotyledon, but as a result of further investigations abandoned that position and adopted a modification of the views of Warming to the effect that the mesocotyl and coleoptyle represent a metamer distinct from the scutellum. The epiblast he held to be a rudimentary second cotyledon. Van Tieghem carried this interpretation to its logical conclusion and adopted the view that the apparent similarity between the grasses and other monocotyledons did not represent homologies, but that the two groups were phylogenetically distinct. He further held, on the strength of anatomical differences, that the portion of the axis between the scutellum and the coleoptyle is in some grasses an internode and in others an enlongated node. The evidence regarding the morphology of the mesocotyl appears so conflicting that a definite interpreta-

[1] For "Literature cited" see p. 301.

tion satisfactory to all morphologists seems very remote. With organs that pertain to the very beginnings of the plant, even the primary differentiation into root, stem, and leaves may not be complete, and to insist on a definite classification of these primitive organs may be idle.

Studies of seedlings of Hopi maize show that the mesocotyl may frequently develop up to lengths of 36 cm.,[1] and it has been possible to note a fact which appears thus far to have escaped notice—namely, that the mesocotyl may give rise to roots at any point on its surface—but these roots are threadlike and do not resemble the roots that arise from the nodes of the culm. They do, however, closely resemble the roots that arise from the radicle immediately below the seed. (See Pl. XXIX, fig. 1.) In grasses roots usually arise from nodes, not from internodes, and the presence of roots on this organ in maize distinguishes it sharply from subsequent internodes and is an argument in support of the interpretation that this intercalary growth, long though it is, is really a part of the cotyledon and may properly be termed a mesocotyl. A further reason for retaining the term "mesocotyl" is because the interpretation implied by its use permits more direct comparisons with other groups of monocotyledonous plants, where the organ sheathing the plumule seems undoubtedly to be a part of the cotyledon.

From observations upon many varieties of maize it has become apparent that when a grain of corn germinates in the ground this usually insignificant organ is of vital importance to the life of the plant, for it is through the elongation of the mesocotyl that the shoot is enabled to reach the surface. So long as the seedling remains below ground, away from light, the mesocotyl will continue to elongate until it reaches a maximum length, which we have found to differ in different varieties, but which seems reasonably constant within the variety. As the mesocotyl elongates, the coleoptyle, with its firm, sharp point, is pushed upward through the soil. As soon as the coleoptyle emerges from the soil, the elongation of the mesocotyl ceases, and elongation of the internode bearing the first true leaf begins, forcing open the coleoptyle.

If the seed is planted so deep that the maximum elongation of the mesocotyl, which in anatomical structure shows a striking relation to the radicle, fails to bring the coleoptyle to the surface, the task of penetrating the soil and reaching light devolves upon the first true leaves. In comparison with the sharp coleoptyle, these leaves are but poorly adapted for forcing their way through the soil, and if the tip of the coleoptyle stops more than a few centimeters below the surface these leaves usually crumple and never reach the light.

In the varieties of maize commonly grown we have been unable to force the mesocotyl to a length greater than 10 cm., while in the Hopi and Navajo varieties this usually minute organ has in our experiments frequently reached the enormous length of 25 or even 30 cm.

[1] In Euchlaena also the mesocotyl may reach a length of 28 cm. Van Tieghem gives 3 cm. as the maximum length of this organ in grasses.

## GERMINATION OF NAVAJO MAIZE

It has been frequently stated that the Navajos, like their neighbors, the Hopi and Zunis, plant maize at unusual depths, 15, 30, and even 45 cm. having been reported. Since planting at such depths is known to be impracticable with other varieties, experiments were planned to test the ability of the Navajo maize [1] to pierce the soil. A representative experiment is here reported. A box 70 cm. long, 33 cm. wide, and 34 cm. deep was sunk in the ground. A quantity of sandy-loam soil sufficient to fill the box was slightly moistened and carefully sifted. At one end the box was filled to within 1 cm. of the top, the soil sloping in a straight line to within 1 cm. of the bottom at the other end.

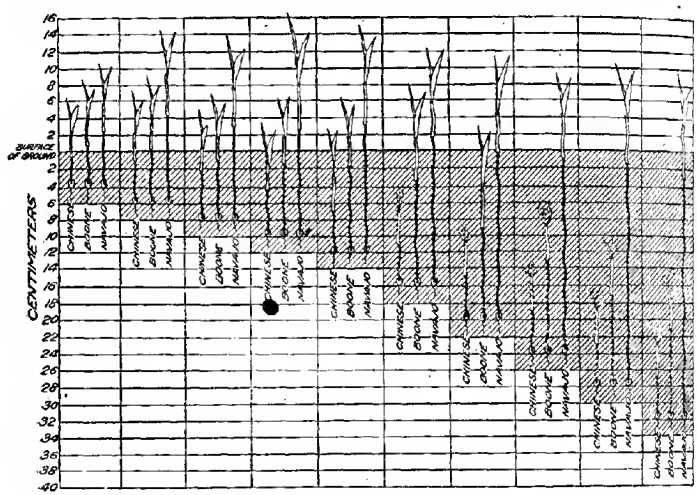



FIG. 2.—Diagram showing the average size of seedlings of Chinese, Boone County White, and Navajo maize planted at different depths.

Five seeds each of Navajo, Boone County White, and Chinese maize were placed in a row transverse to the inclined surface of the soil, 2 cm. from the top of the box. A similar row was planted at a depth of 4 cm. from the top, and so on at the following depths: 6, 8, 10, 12, 16, 20, 24, 28, and 32 cm. The box was then filled with the soil and struck off level with the top. The seeds germinated promptly, and when the most advanced seedlings had reached a total height of about 60 cm. the plants which appeared above the surface were dug up, and the mesocotyl and coleoptyle were measured. (See Table I and fig. 2.)

[1] In the fall of 1912 Messrs. Walter T. Swingle and Karl F. Kellerman visited the region about Shiprock, N. Mex., in the Navajo Reservation and secured specimen ears of the maize grown by the Navajos. This collection was kindly placed at the disposal of the writer. Additional seed was later secured through the courtesy of Mr. William T. Shelton, Indian agent at Shiprock.

TABLE I.—*Average measurements of seedlings of Chinese, Boone County White, and Navajo maize planted at different depths.*

| Depth. | Chinese. | | | Boone County White. | | | Navajo. | | |
|---|---|---|---|---|---|---|---|---|---|
| | Coleoptyle. | Mesocotyl. | Coleoptyle and mesocotyl. | Coleoptyle. | Mesocotyl. | Coleoptyle and mesocotyl. | Coleoptyle. | Mesocotyl. | Coleoptyle and mesocotyl. |
| *Cm.* | *Cm.* | *Cm.* | *Cm.* | *Cm.* | *Cm.* | *Cm.* | *Cm.* | *Cm.* | *Cm.* |
| 2 | 2.3 | 2.3 | 4.6 | 3.7 | 3.2 | 6.9 | 5.5 | 5.0 | 10.5 |
| 4 | 2.5 | 3.5 | 6.0 | 3.1 | 4.9 | 8.0 | 4.3 | 6.5 | 10.8 |
| 6 | 2.8 | 5.0 | 7.8 | 3.4 | 6.1 | 9.5 | 5.2 | 10.2 | 15.4 |
| 8 | 2.5 | 5.8 | 8.3 | 2.8 | 7.4 | 10.2 | 4.9 | 11.0 | 15.9 |
| 10 | 3.2 | 5.8 | 8.9 | 3.1 | 8.6 | 11.7 | 5.6 | 12.2 | 17.8 |
| 12 | 4.0 | 5.2 | 9.2 | 3.4 | 10.4 | 13.8 | 5.0 | 15.1 | 20.1 |
| 16 | ..... | ..... | ..... | 4.6 | 12.4 | 17.0 | 4.3 | 17.5 | 21.8 |
| 20 | ..... | ..... | ..... | 4.5 | 10.9 | 15.4 | 4.7 | 19.7 | 24.4 |
| 24 | ..... | ..... | ..... | ..... | ..... | ..... | 5.2 | 23.0 | 28.2 |
| 28 | ..... | ..... | ..... | ..... | ..... | ..... | 5.6 | 26.5 | 32.1 |
| 32 | ..... | ..... | ..... | ..... | ..... | ..... | 6.5 | 29.0 | 35.5 |

Twelve cm. was the greatest depth from which seedlings of the Chinese variety appeared at the surface. Seedlings of Boone County White appeared from all depths up to 20 cm., while plants of Navajo maize appeared from all plantings, including the very deepest, 32 cm.

There were numerous instances in which the combined length of the mesocotyl and coleoptyle was less than the depth at which the seed was planted. This, of course, means that the upper layers of the soil were penetrated by the true leaves. The maximum depth of soil thus penetrated by the true leaves of the plants of the Chinese variety was 5 cm. One plant of Boone County White maize forced its leaves through 8 cm. of soil. In all of the Navajo plants the coleoptyle reached the surface.

The extent to which the seedlings of the Chinese and Boone County White varieties were able to penetrate the soil by means of the true leaves was doubtless much greater in the carefully prepared soil of the experiment than it would be under field conditions, where any slightly compacted lump of soil would deflect the tender leaves and cause them to crumple. On the other hand, many seedlings failed to come up where there was less than 2 cm. between the top of the coleoptyle and the surface of the ground. The results clearly show that the coleoptyle is the proper organ for penetrating the soil, and where this office devolves upon the leaves there will be many plants that fail to reach the surface.

It has been observed in many field plantings that the spatulate first leaf, formerly called the cotyledon, is the first evidence of the germinating plant. When this occurs in any considerable proportion of the plants, it is safe to assume that the seed has been planted too deep for the best results.

The three types of maize used in the box experiment were also planted in the field. Four seeds of each of the varieties were planted as follows: At the surface and at 5, 10, 20, 30, and 40 cm. below the surface. The greatest depth from which plants of the Chinese variety reached the surface was 10 cm., that of the Boone County White was 20 cm., while that of the Navajo was 30 cm.

The seeds planted at the surface were naturally the first to appear, but on June 17, one month after planting, the largest of the Chinese variety were those from a depth of 5 cm., while the largest plants of both the Boone County White and the Navajo maize were from the 10-cm. depth. On July 11 the plants that came up from a depth of 10 cm. were the tallest in all the varieties, including the Chinese, and to the end of the season this appeared the most favorable depth for the Chinese and Boone County White varieties. With the Navajo, however, the plants from a depth of 20 cm. had equaled those from the 10-cm. depth before the end of July, and from that time the plants from the 20-cm. planting continued to make the most rapid growth, as though this depth represented the most favorable condition for the Navajo variety.

## DESCRIPTION OF ROOT SYSTEM

We have observed further that the root systems of the Navajo, Hopi, and Zuni varieties differ from those of the other varieties; the roots of their seedlings extend to a greater depth, and there is only a single root arising from each seed, while in the seedlings of the Chinese and Boone County White varieties the roots are shorter and more numerous.

The roots of maize are of two kinds: Those that arise from the embryo or seed, called "seminal roots," and those produced from the nodes of the plant. Of the latter class those that arise from the nodes above the ground are often called "brace roots" or "aerial roots." In the varieties commonly grown in the United States there are, in addition to the primary root, or radicle, from two to six additional roots that arise from the base of the cotyledon. These secondary seminal roots, though appearing somewhat later, usually equal or exceed the radicle in size. In the Pueblo varieties of maize these secondary seminal roots have been absent in all seedlings thus far examined, the radicle being the only root arising from the seed. (See Pls. XXIX and XXX, fig. 2.)

## FIELD STUDIES OF PUEBLO VARIETIES OF MAIZE

In September, 1913, opportunity was afforded for a visit to the Zuni, Navajo, and Hopi Indian Reservations of Arizona and New Mexico. It was thus possible to form some idea of the agricultural significance of the peculiar habits of germination of this type of maize.

The value of deep planting made possible by the greatly elongated mesocotyl was obvious. In the localities selected by the Indians for

planting maize the soil is sandy, and in the absence of spring rains the surface layers are, of course, very dry. (See Pl. XXXI, figs. 1 and 2.) The seed, to germinate at all, must be planted deep enough to be in contact with the moist soil. In Navajo fields near Tohatchi, N. Mex., plants were dug up, and the remains of seeds were found at depths ranging from 13 to 18 cm. below the surface. Similar depths were found in a Zuni field near Black Rock, Ariz. (See Pl. XXXI, fig. 1.) In a Hopi field at Polacca, Ariz., near the First Mesa, where the conditions are extreme, the seed had been planted at a depth of 25 cm. (See Pl. XXX, fig. 1.) It thus appears that there is no fixed depth for planting, the custom being to plant deep enough to place the seed in moist soil. If the seed were planted at ordinary depths, germination might be delayed until the latter part of June or the first of July, at which time the rains usually occur; or if the seeds germinated as a result of one of the occasional showers occurring in May, the plants would die from subsequent desiccation.

Like the long mesocotyl, the simple radicle of the Pueblo varieties of maize may be looked upon as an adaptation to the extreme conditions that exist where these types are grown. For six or eight weeks after planting, no rain can reasonably be expected, and during this time the moisture is constantly receding from the surface. By concentrating the energy of the seedling into a single root the latter is forced to greater depths and consequently kept in moister soil than would be the case were a number of seminal roots developed.

Under ordinary conditions, where moisture is distributed through the entire seed bed, the seminal roots become of little importance as soon as the seedling is established and nodal roots have developed. If a half-grown or nearly mature corn plant is carefully dug up, the seminal roots and traces of the seed can still be found, but they are usually dry and shrunken and are obviously of little use to the plant. This was also the condition found in Navajo and Zuni maize fields, though the seminal root was more strongly developed than in the eastern varieties. (See Pl. XXIX, fig. 2.) But in the more extreme conditions existing in the fields near the Hopi villages, where the seeds were planted deeper, it was found that the seminal roots were relatively much larger and were still alive and fresh, making it apparent that they retain their function of supplying moisture and are able to play an important part during the entire life of the plant.

In one Hopi field at the base of the First Mesa the hills of maize were planted about 20 feet apart, with from 10 to 20 plants in a hill. The soil was apparently pure sand washed down by the winter rains and entirely destitute of vegetation other than the planted maize. An average hill dug up in the field was found to contain 15 plants ranging from 60 to 90 cm. in height. (See Pl. XXX, fig. 1.) The remains of the seeds were found at 25 cm. from the surface, and from each seed there

descended a single large seminal root. (See Pl. XXX, fig. 2.) These seminal roots were traced to a depth of 35 cm. and extended even farther down. They were still fresh and densely covered with fine branches. This mass of 15 seminal roots, while less in volume than the nodal roots arising near the surface, was apparently playing an important part in the support of the plants. The mesocotyls connecting the seminal roots with the plants above, while dry on the outside, were filled with live tissue quite unlike the dry and shrunken mesocotyls found in plants of similar age grown under more favorable conditions.

When planted by the Indian methods, the Hopi and Navajo varieties of maize have been found superior to the more improved eastern varieties for these very dry regions. At the time of our visit there was a small field near Keams Canyon that had been planted by eastern methods. The plants were in rows and thinned to one stalk to the hill. There had evidently been a fair germination, but the plants had died without reaching maturity and had produced no seed. At the same time, in the nearest Indian fields at Polacca the plants were dark green and maturing a fair crop, though the season was said to have been unusually dry. (See Pl. XXXI, fig. 3.)

Even under irrigation the somewhat larger strains grown by the Navajos have been found to compare very favorably with eastern types. Several acres of Navajo maize were seen at Shiprock, N. Mex., under irrigation. The fields were very uneven, apparently the result of alkali, but in the better portions the yield was good. The plants were standing about 2 feet apart in the row, the rows 4 feet apart, and nearly every plant was bearing from two to four fair-sized ears. (See Pl. XXXII.)

The ears from 36 plants, representing a number of distinct types, were collected. The 36 plants bore in all 94 ears, weighing 37.6 pounds, an average of 15.2 ounces per plant. The plants producing these ears averaged only a little over 5 feet in length.

## CONCLUSIONS

Throughout the western part of the Great Plains area the difficulty of securing uniform germination is a serious obstacle to the growing of maize. With the varieties commonly grown, if the seed is planted at the customary depth, many seeds fail to germinate from insufficient moisture; if planted deep enough to come in contact with moist soil, the plants may fail to reach the surface.

The agricultural Indians of the Southwest have continued from prehistoric times to grow maize successfully in regions where drought, and especially the absence of spring rains, makes it much more difficult to start the crop than in the Great Plains. A study of the varieties grown by the Hopis and other agricultural Indians shows that these varieties possess two special adaptations: (1) A greatly elongated mesocotyl that permits deep planting and (2) the development of a single large radicle

that rapidly descends to the moist subsoil and supplies water during the critical seedling stage.

This indigenous type of maize seems to have attracted little attention, perhaps because it has been included in the popular mind with a series of inferior varieties commonly known as "squaw corn." But the Pueblo Indians of Arizona and New Mexico have strains sufficiently productive to compare favorably with improved varieties even when grown under irrigation. The peculiar adaptations of this type definitely indicate its value for the semiarid regions and warrant experiments to determine the possibility of its utilization.

## LITERATURE CITED


BRUNS, ERICH.
1892. Der Grasembryo. Flora, Jahrg. 76, p. 1–33.

ČELAKOVSKÝ, L.
1897. Über die Homologien des Grasembryos. Bot. Ztg. Jahrg. 55, p. 141–174.

GOEBEL, K. E.
1905. Organography of Plants. Pt. 2, Oxford, p. 416.

HACKEL, EDUARD.
1897. Gramineae. Engler, Adolf, and Prantl, K. A. E., Die Natürlichen Pflanzenfamilien. T. 2, Abt. 2, p. 10.

HEGELMAIER, FRIEDRICH.
1874. Zur Entwicklungsgeschichte monokotyledoner Keime nebst Bemerkungen über die Bildung der Samendeckel. III. Bot. Ztg. Jahrg. 32, col. 661.

HOFMEISTER, WILHELM.
1858. Neuere Beobachtungen über Embryobildung der Phanerogamen, Jahrb. Wiss. Bot. [Pringsheim] Bd. 1, p. 154.

HOLM, THEODOR.
1908–9. Observations on seedlings of North American Phaenogamous plants. Ottawa nat. v. 22, p. 165–174, 1908; p. 235–244. 1909.

KLEBS, GEORG.
1885. Beiträge zur Morphologie und Biologie der Keimung. Untersuch. Bot. Inst. Tübingen, Bd. 1, p. 536.

RICHARD, L. CL.
1811. Analyse botanique des embryons Endorhizes ou monocotylédonés, et particulièrement de celui des Graminées. Ann. Mus. Hist. Nat. [Paris], t. 17, p. 223–251; 442–487.

SACHS, JULIUS.
1875. Text-book of Botany. Oxford, p. 541.

SCHLICKUM, AUGUST.
1896. Morphologischer und anatomischer Vergleich der Kotyledonen und ersten Laublätter der Keimpflanzen der Monokotylen. Stuttgart, p. 56. (Bibliotheca Bot. Heft 35.)

VAN TIEGHEM, PHILIPPE.
1872. Observations anatomiques sur le cotylédon des graminées. Ann. Sci. Nat. Bot. s. 5, t. 15, p. 236–276.
1897. Morphologie de l'embryon et de la plantule chez les graminées et les cypéracées. Ann. Sci. Nat. Bot. s. 8, t. 3, p. 259–309.

WARMING, EUG.
1879–80. Forgreningen og Bladstillingen hos Slaegten *Nelumbo*. [Footnote.] Vidensk. Meddel. Naturhist. Forening. Kjøbenhavn, p. 446–448.

## DESCRIPTION OF PLATES

PLATE XXIX. Fig. 1.—A seedling of Hopi maize with mesocotyl 18 cm. long. The seed was planted in sand 20 cm. below the surface. There is a single seminal root with threadlike branches similar to those arising from the mesocotyl. The first nodal roots have begun to form at the base of the coleoptyle. One-half natural size.

Fig. 2.—The root system of a plant of Zuni maize dug from a field near Zuni, N. Mex., showing the well-developed, single seminal root and the comparatively feeble nodal roots. Natural size. The field from which this plant was dug is shown in Plate XXXI, figure 1.

XXX. Fig. 1.—A hill of Hopi maize containing 15 plants grown under conditions of extreme drought at the base of the First Mesa near Polacca, Ariz. The ears can be seen borne at the surface of the ground.

Fig. 2.—A plant of Hopi maize. One of the smaller plants from the hill shown in figure 1. The remains of the seed are scarcely visible at the sharp bend of the mesocotyl, 25 cm. below the surface of the ground.

XXXI. Fig. 1.—A field of Zuni maize near Zuni, N. Mex. One of the hills near the center containing but a single plant shows a relatively large ear borne at the surface of the ground.

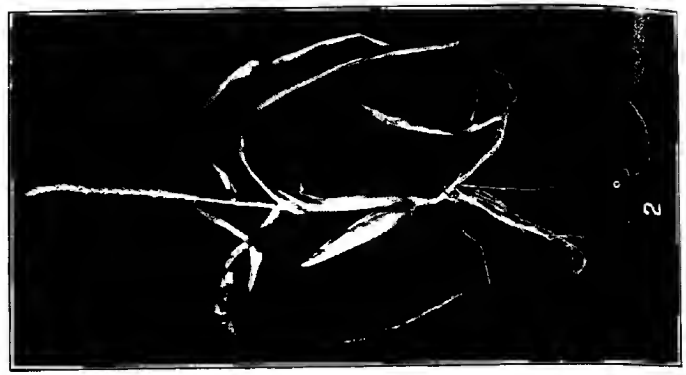
Fig. 2.—A hill of Zuni maize in the field shown in figure 1. Note the large ears borne near the surface of the ground.

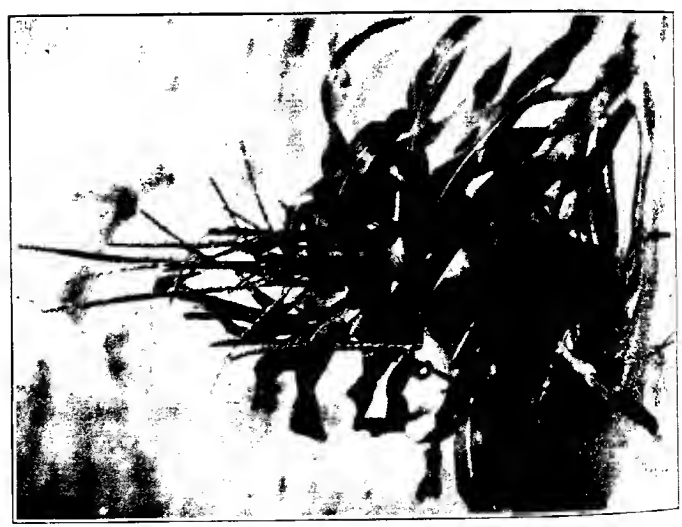
Fig. 3.—A hill of Hopi maize making luxuriant growth under conditions of extreme drought. Note the manner in which the low-spreading plants shade the ground. Polacca, Ariz.

XXXII. Fig. 1.—A single plant of Navajo maize grown under irrigation at Shiprock, N. Mex.

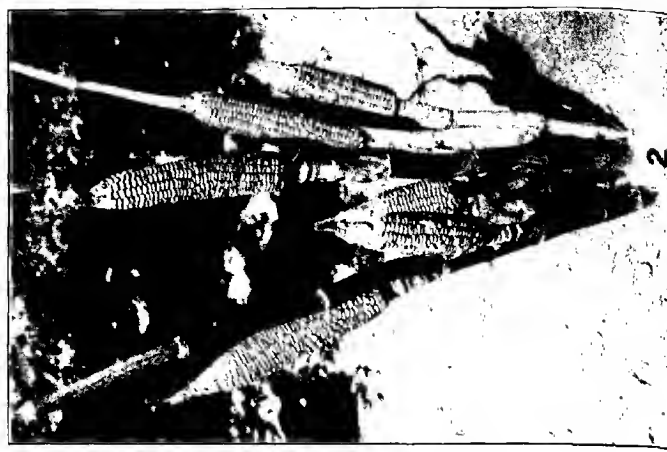
Fig. 2.—The basal portion of the plant of Navajo maize shown in figure 1, with leaves and husks removed. The ears from this plant after drying weighed 2 pounds.

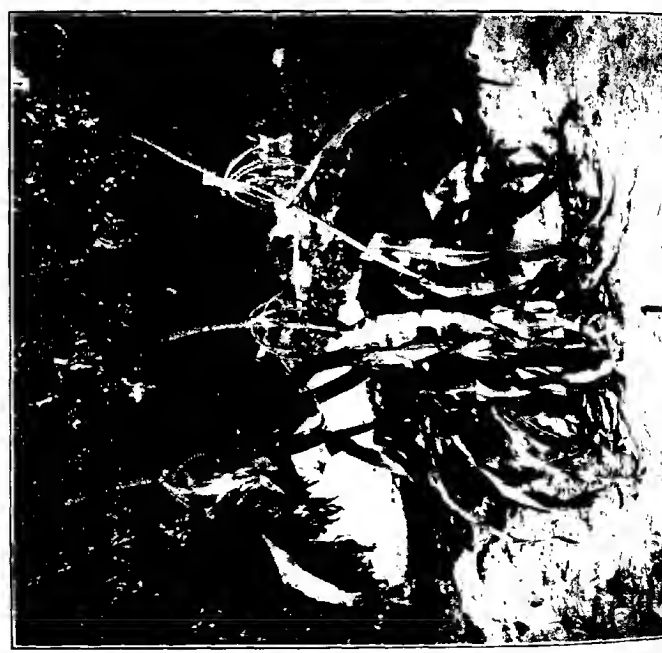

1
2






1
2
3


2


1

# SOME DISEASES OF PECANS

By FREDERICK V. RAND,
*Scientific Assistant, Fruit-Disease Investigations, Bureau of Plant Industry*

## INTRODUCTION

The pecan, *Carya illinoensis* (Wang.) K. Koch,[1] is an indigenous tree of the hickory group, which has long been famous for the excellent quality of its fruit. From the time when the earliest settlers first gathered the nuts from native forest trees the pecan has been growing steadily in favor.

Until recently the entire supply has come from the wild forest trees and from a comparatively few, more or less isolated seedling orchards. During the last 15 years, however, artificial propagation by budding and grafting has gradually assumed a commercial importance until at the present time a large number of excellent horticultural varieties are available. These are being planted on a large commercial scale and through an ever-widening range.

The pecan is found native on low, rich ground in the neighborhood of streams from the valley of the Mississippi River in Iowa through southern Illinois and Indiana, western Tennessee to central Alabama and Mississippi, western Louisiana through Arkansas and Missouri to southeastern and western Kansas, eastern Oklahoma, and the valley of the Concho River, Tex. It is also found in some of the mountain regions of Mexico. As a native tree the pecan is most abundant and attains its largest size in southern Arkansas, eastern Oklahoma, and middle to eastern Texas.[2] As a cultivated tree, however, it is by no means confined to the sections above enumerated. Plantings of greater or less extent have been made in Virginia, North Carolina, South Carolina, Georgia, Florida, New Mexico, California, Oregon, and Washington, with small experimental plantings in several other States.

Notwithstanding the highly colored statements of some of the early promoters of pecan culture, this tree, like all of our cultivated fruit trees, has its insect and fungous enemies. Possibly they would form a shorter list than would those of some of our common fruits, but they are none the less real and important, for, whenever a plant is brought under cultivation or taken out of its native range, new diseases and new problems with old diseases are sure to follow.

Other things being equal, the larger the number of individuals of a host species growing in a given area the greater the chances any particu-

[1] Synonyms: *Carya olivaeformis* Nutt.; *Hicoria pecan* Brit.; *Juglans pecan* Marsh.
[2] Sargent, C. S. Manual of the Trees of North America. Boston, 1905. p. 134

lar parasite has of successfully reproducing itself from season to season, and consequently the more general and severe will be its injury over that area. Thus, a disease occurring occasionally or with but slight injury upon more or less isolated host individuals may under conditions of close orchard planting assume an entirely different aspect, becoming more nearly seasonal in its occurrence and causing a much greater percentage of injury. A large part of the assumed difference in injury by a disease under native and under orchard conditions is, however, often merely psychological. In orchard culture the ideal sought is a thrifty growth and abundance of high-grade fruit for every tree planted. Any deviation from this ideal is quickly noted by the grower; whereas little consideration is given to the facts that under native conditions large numbers of individuals succumb to disease for every one that persists and reaches maturity and that careful observations and comparisons are seldom made with those which do reach maturity.

Nevertheless, the general fact remains that well-known diseases are often more destructive under orchard conditions. Further than this, diseases of hitherto unknown occurrence upon a particular host may suddenly make their appearance. Some of these may have been present but previously unnoticed, while others may be actually new to the host. They are often brought to a locality with the introduction of new plants, and with the widening of the range of a host the diseases of related plants will be encountered sooner or later. Furthermore, a parasite is often more destructive when brought to a new locality, either because of the absence of its former enemies or because of other conditions more favorable to its growth and reproduction in the new environment.

It has long been known that where a considerable number of plants or animals are exposed in a similar way to the attacks of a parasitic disease more or less difference will be noted in their behavior toward the disease. In many cases some individuals will be found which seem to be entirely immune, others which are very susceptible to attack, and still others with varying grades of immunity or susceptibility between two extremes. In localities favorable to the growth and spread of a disease this condition works for the general benefit of the species attacked. Those individuals least susceptible to injury will be most successful in reproducing themselves, and thus a more or less immune race will be developed. On the contrary, if a race has arisen amid conditions unfavorable to the development of a particular disease, or in its entire absence, growth and reproduction will have taken place with little or no relation to the disease. If such a race is exposed to the disease, it is probable that a large percentage of its individuals will be found to be susceptible.

These relations between host and parasite, though only a few among many, may at least serve to indicate the extreme complexity of all prob-

lems having to do with living things. Partly because of this complexity most problems of disease control are problems of "better and worse" rather than of "good and bad," for very few varieties prove to be absolutely immune, and very few artificial methods of control are entirely effective.

The present paper deals only with certain distinct and more or less troublesome fungous and bacterial diseases of pecans.[1] For the most part these studies were carried on during the years 1911 and 1912.

## NURSERY-BLIGHT

[Caused by *Phyllosticta caryae* Peck]

### HISTORY AND DISTRIBUTION

Nursery-blight is one of the worst known diseases of the pecan to affect nursery seedling trees. However, in spite of the fact that young trees are often defoliated from this cause by midsummer, no definite investigation has hitherto been carried out and published, so far as could be ascertained. This may be due partly to the fact that the pecan nursery business is of comparatively recent origin and partly to the obscurity of the causal fungus.

The distribution of this disease has been found to correspond very closely with that of the pecan scab and the brown leaf-spot. Affected specimens have been received from most of the pecan-growing States, and personal observations have further demonstrated its presence at Petersburg, Va.; Orangeburg, Summerton, and Charleston, S. C.; Albany, De Witt, Hardaway, Baconton, Thomasville, and Cairo, Ga.; Tallahassee, Newport, Monticello, Glen St. Mary, Jacksonville, St. Augustine, Palatka, and Belleview, Fla.; New Orleans, La.; and at San Antonio, Boerne, Kerrville, Waco, and San Saba, Tex. Strains of the fungus obtained from as widely separated points as Florida and Texas have been similar in cultural characters and have caused the same symptoms upon artificial inoculation, thus demonstrating the disease in both cases to be of the same origin. Wherever observations have been made the disease has for the most part been found to affect young trees, and by far the greatest injury has been to the 1 and 2 year old nursery stock.[2] Mature trees are seldom seriously injured.

[1] No discussion of the scab, a serious disease of pecans, is included in the present paper.

[2] A very effective control of the nursery-blight with Bordeaux mixture was obtained in two different localities during the season of 1911, and there appears to be little reason to doubt that it will prove efficacious in other localities and seasons. The quantity of spray material used and the cost of application under nursery conditions are small, and it is thought that the increase in size and vigor, together with better conditions for budding, will amply repay the small cost in material and labor necessary for the treatments. It is obvious that the first application should be made before the disease has gained much headway in the spring. Three to five subsequent applications may then be given at intervals of three to four weeks, according to the season.

### SYMPTOMS OF THE DISEASE

So far as has been observed nursery blight affects only the leaf blade, but infections occur from early spring well on through the season, so that under conditions favorable to the development of the disease the young trees have little opportunity for growth. Generally the first indications of infection appear in the form of minute roundish spots, which are dark reddish brown on the upper leaf surface and blackish on the lower. (Pl. XXXVII, Fig. *C*.) These slowly increase in size until a diameter of 2 to 5 mm. is often reached in the individual spots. With increase in size the center of the spot on the upper surface assumes an ashen-gray color, which is usually bordered with reddish brown, while the lower surface remains black throughout or with an occasional tiny ashen-gray spot in the center of this dark-colored area. (Pl. XXXVII, Fig. *I*.) The gray color in both cases is caused by a raising of the epidermis, thus leaving an air space between it and the tissues

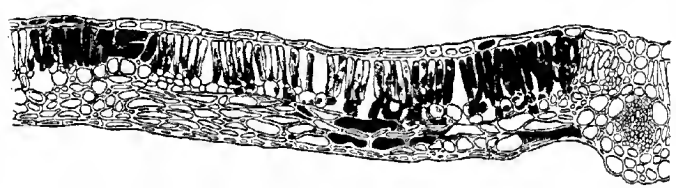


FIG. 1.—Cross section of pecan leaf recently infected with the nursery-blight fungus (*Phyllosticta caryae* Peck) from pure culture. ×260.

below. The leaves are often considerably peppered with these spots, and by their coalescence larger areas are often involved. Very frequently the spots elongate and coalesce along the midrib and larger veins, thus giving a very characteristic appearance. The parenchyma cells and vascular bundles are often killed and discolored over large areas. Whenever the vascular tissue becomes involved to any great extent the supply of water is cut off from below and the leaf soon dries up and falls. Figure 1 shows the microscopical appearance of the diseased cells in a recently infected leaf.

### MYCOLOGICAL AND PATHOLOGICAL STUDIES

#### ISOLATION OF THE FUNGUS

Rough microscopical examination of a considerable range of diseased material disclosed no fungous or bacterial form which was at all constantly associated with the symptoms. Occasionally a tiny thin-walled pycnidium was encountered, but no spores were found and usually no fungous mycelium or fruiting body of any kind. Cultures made during 1910 and 1911 from material several days old gave only saprophytic fungi as shown by the negative results of all the inoculation tests.

Experimentation had already shown that the disease was readily controllable by Bordeaux mixture, and hence it was thought highly probable that it was of parasitic origin. Consequently, in the summer of 1912, materials for making cultures were taken directly into the field with the idea of locating the cause, if possible, by any of the ordinary methods of isolation. Leaves showing very recent infection were taken from the highest parts of the trees where there was little or no spattering from the soil. These leaves were placed in sterile Petri dishes and taken to the temporary laboratory, where the tiny spots were cut out at once with sterile scissors and transferred by the ordinary poured-plate method to Petri dishes of corn meal and synthetic agar.[1] After 24 to 48 hours colonies became visible which had evidently originated from the diseased areas, and their appearance was quite uniform in all the cultures, except in a few cases where contaminations had entered. Transfers were then made to tube cultures. In this way strains of the fungus were obtained from Monticello, Fla., from Albany, Ga., and from San Antonio, Waco, and San Saba, Tex.

### Inoculations

Circumstances connected with field travel prevented the making of any inoculation tests during the summer of 1912, but the following summer and winter trials were carried out upon pottted seedling trees in the greenhouse. The trees were sprinkled, inoculated from pure cultures, and covered after inoculation for several days with bell jars. Three strains of the fungus were used in this work: One from Texas, one from northern Florida, and a strain reisolated from an artificially infected leaf.

Experiment No. 1 (Oct. 8, 1912).—The young leaves on four trees were inoculated from 1½-months-old, nonsporiferous synthetic-agar cultures (Florida strain 122), the slimy mycelial mass being smeared over portions of both leaf surfaces. These four trees and two moistened but uninoculated check trees were left under bell jars for five days. After a week small dark-brown specks were noted over the inoculated areas. In three weeks these spots were 1 to 2 mm. in diameter and in every way similar to natural infections. The check trees remained uninjured.

Experiment No. 2 (Nov. 9, 1912).—The young leaves on three seedlings and the matured leaves on two others were inoculated as above only from 3-weeks-old, sporiferous corn-meal-agar cultures (Florida strain 122). The five inoculated and five check trees were left under the bell jars for three days. Observation after two weeks showed the production of small, roundish, dark-brown specks, which at three weeks had become 1 to 3 mm. in diameter with small ashen-gray areas in the center. The lower

[1] Synthetic agar.—(1) 1,500 c. c. of distilled water and 36 grams of agar. Cook in double boiler for one hour at 15 pounds pressure.

(2) 500 c. c. of distilled water, 200 grams of dextrose, 40 grams of peptone, 20 grams of ammonium nitrate, 5 grams of magnesium sulphate (crystals), 10 grams of potassium nitrate, 5 grams of potassium acid phosphate ($K_2HPO_4$), and 0.2 gram of sodium chlorid.

Boil in double boiler for 30 minutes, add agar and cook for five minutes. Restore to volume, titrate, cool to 60° C., and add whites of two eggs. Cook to coagulate eggs, filter, tube, and sterilize.

This formula is modified from that given by Francis Darwin and E. Hamilton Acton in their Practical Physiology of Plants, ed. 3, 1901, p. 68.

surface of the infected areas was almost black. Infection had taken place upon all the leaves inoculated, while none of the check trees showed any signs of the disease.

EXPERIMENT NO. 3 (Dec. 7, 1912).—The mature leaves of four seedlings were inoculated from 3-weeks-old, sporiferous corn-meal-agar cultures (Florida strain 122), and those of three other seedlings from nonsporiferous synthetic-agar cultures of the same age and strain. The seven inoculated plants and four checks were kept under bell jars for three days. Observations at two weeks showed the leaves of the first set with tiny dark-brown specks scattered over the inoculated areas and with some of the spots beginning to show the grayish centers. The leaves inoculated from the synthetic-agar cultures were similar, but not quite so far advanced. The check trees all remained uninjured.

EXPERIMENT NO. 4 (Dec. 7, 1912).—The mature leaves of three seedlings were in like manner inoculated from 3-weeks-old, sporiferous corn-meal-agar cultures (Texas strain 127). At the end of one week the spots were just becoming visible, and after two weeks the centers were turning gray on the upper surface, while the borders remained the typical dark brown. There were no evidences of the disease on the two check trees. All five trees had been covered with bell jars for the first three days.

EXPERIMENT NO. 5 (Dec. 18, 1912).—Two trees were inoculated from corn-meal-agar cultures isolated from one of the trees of experiment No. 3 (strain 163). Typical infections appeared at five to seven days, and these gradually increased in size for three weeks, finally taking on the grayish center and dark reddish brown border above, with the color almost black below. The check trees remained healthy.

EXPERIMENT NO. 6 (Dec. 18, 1912).—The mature leaves of three seedlings were inoculated from sporiferous corn-meal-agar cultures of two weeks' incubation (Florida strain 122). In this case the pycnidia were broken up in sterile distilled water and sprayed upon the leaves. The three check trees were sprayed with sterile distilled water, and all six trees were left under bell jars for three days. On removing the bell jars it was noted that tiny dark-colored specks were forming over much of the areas inoculated. These later proved to be the typical spots of the nursery-blight. No evidence of disease appeared on the check trees.

EXPERIMENT NO. 7 (Dec. 23, 1912).—The sporiferous pycnidia from young corn meal-agar cultures (Florida strain 122) were broken up in sterile distilled water and sprayed upon the upper and lower surfaces of the leaves of three seedling pecan trees, the leaves having previously been washed. Three days after inoculation sample inoculated and check leaves were collected. These were killed and bleached in alcohol, stained with eosin, and examined superficially under the microscope. The conidia themselves, being almost bacillar in size, could not be seen with the low power necessary in any such examination. However, here and there could be distinguished a very fine mycelial growth stained pale pink by the eosin, and in a number of cases hyphæ were clearly seen entering the leaf through stomatal pores or openings left by the breaking off of leaf hairs and resin glands. In one case the branching hypha could be followed some distance beneath the epidermis from the stoma through which it had entered. The check leaves showed no such fungous growth entering the leaf.

After a week an examination of the leaves left on the trees showed tiny dark-colored spots scattered over the inoculated areas, while at two weeks the typical grayish centers had developed. The check leaves were still without injury.

In the above detailed experiments the leaves of 24 pecan seedlings were inoculated at different stages of maturity and with three strains of the fungus. Every inoculation was successful, and in no case did any of the check trees show signs of the disease. These data seem to establish the parasitism of the fungus beyond any doubt.

From the facts that most of the infections occur within 2 or 3 feet of the soil surface, that such infections may take place through stomata and other openings in the epidermis, and that pycnidia are of rare occurrence upon the leaves while still attached to the tree, it seems very likely that the general development of pycnidia takes place upon the dead and decaying leaves after they have fallen to the ground and that most of the infection occurs through the spattering of spore-bearing material from the soil.

## Cultural Studies

### Thermal tests

Four series of thermal tests were carried out, corn-meal-agar cultures being incubated for two to three weeks at temperatures ranging from 1° to 40° C. No change occurred at 1° or at 40°, while at 5° and 36° growth, where it occurred at all, was so small as to be scarcely discernible. The growth of the colony was extremely slow at 8°, but increased considerably in rate at 12°. At 14°, 16°, and 20° the rate was nearly the same, though with a very gradual increase toward the higher temperature. The optimum for the temperatures tested occurred at 30°, while at 32° growth was very similar to that at 12° to 14°. Incubation of two or three weeks at 37° to 40° invariably killed the fungus, no subsequent growth taking place when again held at optimum temperatures.

Thus, incubated in corn-meal-agar slant tubes, the fungus made at least some growth at temperatures ranging from 5° to 36° C. (41° to 97° F.), with a very gradual decrease in rate from the optimum (30° C. or 86° F.) downward, and a rather rapid decrease upward. The comparatively high optimum temperature, together with the wide range of effective growth at lower temperatures, will assist in explaining the extended and continuous period of infection observed under field conditions.

### Cultural characters

The more obvious characters of the fungus as grown upon a number of culture media are as follows:

Beef-Agar Slant Tubes.—The colonies are at first somewhat convex, pale ocherous in color, with slightly roughened but glistening surface, and without aerial mycelium. Later, the surface becomes much wrinkled, often presents a corallike growth in the older parts, and approaches a light Venetian red in color. A moderate production of pycnidia usually takes place in cultures 1 or 2 months old. Colonies often attain a diameter of 10 to 12 mm.

Corn-Meal-Agar Slant Tubes (Pl. XXXVII, fig. *H*).—Where little aerial mycelium is present, the colonies are at first about the same color and general appearance as in the young beef-agar cultures. The cottony aerial mycelium becomes a faint pinkish white and is often present in considerable luxuriance. The submerged parts sometimes give a pale-violet tinge to the agar, but little or no direct diffusion of color into the medium has taken place. Pycnidia are produced in abundance and range from 75 to 150 $\mu$ in diameter. At first they are a pale-ocherous color, but later change to dark brown or almost to black. Many cross connections between the hyphæ have

been observed, and swollen cells are commonly scattered here and there through the mycelium. Colonies often cover the slant, but unlike those on beef agar they are seldom much wrinkled.

Corn-Meal Flasks.—On this medium the colonies with 1 or 2 months' growth attain a diameter of 5 or 6 cm., and become deeply convoluted or wrinkled. The cottony aerial mycelium where present is similar in color to that on corn-meal agar, while the underlying pseudoparenchyma en masse takes on a yellowish burnt-sienna tinge. Pycnidia were not observed.

Filter Paper.—Growth on filter paper moistened with sterile distilled water gave small colonies of a pale-violet color and with or without a scant pinkish white aerial mycelium.

Oxalic-Acid-Agar Slant Tubes.—The colonies are raised to convex, pale ocherous around the margin and approaching a sepia brown throughout most of the central portion. With age the vegetable dye of the medium becomes bleached, so that the color of ordinary beef agar is finally assumed. No pycnidia were observed.

Synthetic-Agar Slant Tubes[1] (Pl. XXXVII, fig. *G*).—The colonies are very convex, with moist and glistening surface. The mycelial mass is extremely viscous, much convoluted, burnt sienna to brown in color, and with the drying out of the cultures assumes various shades of olive green, violet, brown, and reddish brown. Numerous cross connections between the hyphæ were noted, but no pycnidia have yet been developed on this medium.

## Morphology and Taxonomy

Several of the diseased spots from fresh material were killed in Carnoy's fluid, embedded in paraffin, and sectioned both vertically and horizontally in order to locate the course of the fungous growth within the tissues. The mycelium was found to be septate, very fine, and nearly or quite hyaline; and even in the stained vertical sections it was often distinguishable with difficulty. This readily explains the fact that examination of rough mounts from field material rarely gives any evidence of fungous growth within the leaf tissues. The mycelium was best located in the stained horizontal sections, where it could be distinctly seen ramifying through the intercellular spaces just above the lower epidermis and throughout the mesophyll tissue. (Fig. 2.) Where the spots involved the vascular tissue, the hyphæ were often seen extending immediately parallel to the vessels, the latter in such cases being dead and discolored. In many cases this intercellular mycelium had developed scattered swollen cells with large vacuoles, but thus far no definite formation of pycnidia has been observed upon artificially infected leaves. Upon field material, however, the tiny dark-colored fruiting bodies are occasionally encountered upon the upper leaf surface.

In 1887 a Phyllosticta occurring on *Carya alba* was described by Peck,[2] which from his description and an examination of type specimens, appears to be identical with the nursery-blight fungus. Peck's description is as follows:

[1] For the formula for preparing synthetic agar, see p. 307.

[2] Peck, C. H. Plants not before reported. 40th Ann. Rpt., N. Y. State Mus. Nat. Hist., 1886, p. 57, 1887.

*Phyllosticta caryae*, n. sp.—Spots large, irregular, often confluent, at first yellowish, then brown, sometimes becoming grayish in the center; perithecia minute, .004 inch broad, punctate, epiphyllous; spores irregularly elliptical, .0002 inch long, .00008 broad.

Living leaves of hickory, *Carya alba*, Piffard, August.

Several months afterwards Ellis and Everhart[1] described under the same name a Phyllosticta occurring on species of Carya at Newfield, N. J. On account of Peck's priority, the specific name of Ellis and Everhart's fungus was later changed by Saccardo to *caryogena*.[2]

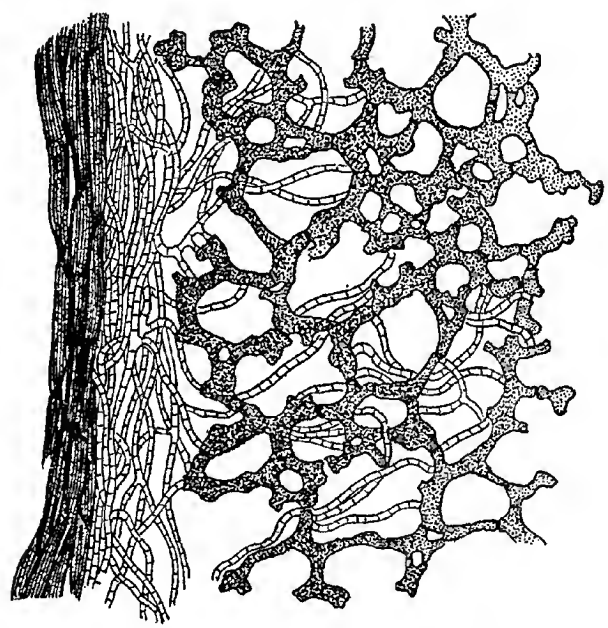


FIG. 2.—Horizontal section of leaf recently infected with the nursery-blight fungus in pure culture. ×150.

After examination of Peck's material the two species were finally considered by Ellis and Everhart as identical, and the following description and statement was published:

*Phyllosticta Caryae* Pk. 40th Rep. 57. 1887.
*P. Caryae* E. & E. Journ. Mycol. 101. 1888.
*P. caryogena*. Sacc. Syll. 10:119. 1892.
Exsicc. Ell. & Evrht. N. A. F. 2155, 2677.
On various species of *Carya* from Maine to Kansas.

Spots large, irregular, often confluent, often acute at each end, with a nerve of the leaf running through the center, .5-1 cm. diam., yellowish at first, becoming brown,

[1] Ellis, J. B., and Everhart, B. M. New species of fungi from various localities. Jour. Mycol., v. 4, no. 10, p. 101, 1888.

[2] Saccardo, P. A. Sylloge Fungorum. v. 10, Patavium, 1892. p. 119.

with the margin darker. Perithecia epiphyllous, minute, lenticular, black-brown, 100 μ broad. Sporules oblong or ellipsoid-oblong, 5—8×2—2.5μ. The fungus is also found on old insect-galls on the same leaves. The 40th Rep. was given to the public in May, 1888. P. *Caryae* E. & E. was not published till October, 1888. P. *Caryae* Pk. and P. *Caryae* E. & E. are evidently the same [1]

The leaf spots upon the pecan assume the reddish brown color at a very early stage of development, though this is often preceded by a slight yellowing of the tissue at the point of infection. Furthermore, the grayish center is almost invariable in its appearance during the later stages. Individual spots have rarely been found by the writer to exceed 4 or 5 mm. in diameter, but by the coalescing of several initial infections diseased areas at least up to 8 or 10 mm. have frequently been observed.

The majority of the pycnidia have been found to vary but little from 100μ in diameter, but extremes of 50 to 150μ have been noted for mature pycnidia in culture. In the latter case they are usually much lighter in color than on the host, assuming macroscopically a tawny appearance. On the pecan leaf and occasionally in culture the fruiting bodies are dark brown to black.

Conidia as observed on this host have corresponded closely with Peck's fungus, ranging within the limits of 3.8 to 6 by 1.5 to 2μ. In other points also the pecan fungus corresponds closely with the two descriptions quoted above.

Thus, on account of the close relationship between the hosts and the many points of resemblance between the fungi and the disease symptoms, it seems best to consider the nursery-blight fungus as identical with *Phyllosticta caryae* Peck rather than to burden mycological literature with another name. At least this course should be followed until cultural and cross-inoculation work can demonstrate a specific difference.

## BROWN LEAF-SPOT

[Caused by *Cercospora fusca*, emend. sp.]

### HISTORY AND DISTRIBUTION

With the growth of the pecan industry the brown leaf spot has gradually been receiving more notice among orchardists. Since it is by no means as serious a trouble as the pecan scab, it has not merited the attention given the latter. No published record has been found, except a brief description of the fungus, and no work establishing the cause or demonstrating a method of control.[2] However, next to the pecan scab it is perhaps the worst and most generally distributed leaf-spot disease

[1] Ellis, J. B., and Everhart, B. M. The North American Phyllostictas. Vineland, N. J. 1900. p. 35.

[2] The brown leaf spot has occurred to a limited extent at points where spraying tests were being carried out on other pecan diseases and has been effectively controlled with three treatments of Bordeaux mixture.

affecting the mature trees and consequently has been considered worthy of investigation as well from a practical as from a mycological standpoint.

For several years specimens of leaves showing this disease have been received from widely different parts of the pecan-growing territory, while within the last two years the writer has made personal observations in the field over much of this region. From these observations and studies in field and laboratory it may definitely be said that the brown leaf-spot occurs in South Carolina, Georgia, Florida, Alabama, Louisiana, and Texas and that an exceedingly similar if not identical disease has in numerous instances been seen on other species of hickory. Furthermore, there is little doubt that its range is much greater than that above indicated, since it has been found in nearly every pecan section visited by the writer during the last three years.

Observations in several States during the past two years have shown very little difference in resistance to the disease among the different varieties. For example, in one orchard examined, containing 45 varieties of the pecan, the brown leaf-spot was so uniformly distributed that no appreciable difference in the amount of injury could be detected among the different varieties. From a number of such observations over a wide territory it may be safely assumed that little difference in resistance exists among the varieties now commonly planted.

## SYMPTOMS OF THE DISEASE

The leaf blade is the only part of the tree known to be affected. (Pl. XXXVII, fig. *A*.) In ordinary seasons or when only a few spots occur, there is little or no appreciable injury, but occasionally under conditions very favorable to the progress of the disease partial defoliation may result. Infections occur from the early part of summer on until fall, and under proper conditions of moisture and temperature even well-matured leaves may develop the disease. Several days after infection (ordinarily 3 to 10) the condition becomes evident through the formation of a tiny dark reddish brown spot, which is usually somewhat angular in outline and bounded by the veins of the leaf. The spots from the earliest visible stages extend through the leaf tissue and appear about the same in form and color on both surfaces. The size increases gradually until the diseased areas often attain a diameter of 10 or even in some cases 15 mm. With increase in area the spot often loses its angular outline and the margin becomes more indefinite, while at the same time the center of the spot may in some cases assume a somewhat lighter reddish brown color with the darker brown as a border. Very often, however, the spots remain angular and with definite margin, though in such cases they seldom attain a diameter of more than 2 or 3 mm. Microscopical examination showed the cells within the affected areas to be dead, more or less opaque, and brownish in color. (Fig. 3.)

## MYCOLOGICAL AND PATHOLOGICAL STUDIES

### Isolation of the Fungus

Examination of a wide range of material during the last three years has invariably shown the same type of fungous growth and spore formation, while no other fungi have been found, except in the later stages of the disease. It was considered very probable that the fungus above mentioned was the cause of the diseased condition which it accompanied, and so on October 4, 1911, single spore cultures were started, using conidia from material collected August 29, 1911, at Baconton, Ga. Synthetic agar was used, and the germination was followed under the microscope from day to day. Growth was rather slow at first, but continued until at the end of a month colonies 5 to 15 mm. in diameter had been formed. From one of the strains obtained in this way the first inoculation tests were made.

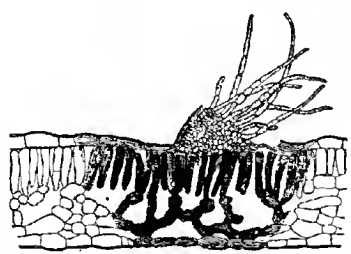


Fig. 3.—Cross section of a leaf infected with the brown leaf-spot fungus from pure culture. ×250.

### Inoculations

The inoculation work was carried out during the winter and spring of 1912 upon young seedling pecan trees in the greenhouse. The leaves to be used in the tests were moistened with water immediately preceding inoculation, and since no definite spore formation has taken place in culture, bits of the mycelial growth were placed directly on the upper or lower sides of the leaves thus moistened. The small potted trees were then generally left for several days under bell jars, with slight ventilation at the base, to insure proper humidity for growth of the fungus. Check trees in each experiment were treated similarly, with the exception of the inoculation.

Experiment No. 1 (Feb. 29, 1912).—The young leaves of two potted seedlings were inoculated from 3-weeks-old oxalic-acid and synthetic-agar cultures (strain 33), the first tree being covered with a bell jar and the second left open. Two check trees were placed under a bell jar. The inoculated and check trees were all sprinkled with tap water on the second and fifth days and the bell jars were removed on the latter date. At the end of two weeks most of the inoculated leaves on the first tree had developed small, reddish brown areas from mere angular flecks up to irregularly circular spots 1 mm. in diameter. Very little infection had occurred on the tree left uncovered after inoculation, but several distinct spots were noted. Later, many of the spots had increased in size up to 7 or 8 mm., with the development of tawny clusters of conidia visible to the naked eye upon the upper leaf surface. In no case did the check trees show signs of the disease.

Experiment No. 2 (Apr. 16, 1912).—In a similar manner the tender leaves of a seedling were inoculated on both surfaces from a month-old corn-meal-agar culture (strain 33). This tree and the check were left under a bell jar for five days. Observation after a month showed a large number of the somewhat angular young spots up

to 1 mm. in diameter, but no spore formation had as yet occurred. After two months the spots were well scattered over the inoculated areas, and some of them had attained a diameter of 10 mm. The pale tawny conidial tufts were at this time very abundant on the upper surface. No infection had taken place on the check tree. After three months many of the smaller spots had coalesced to form reddish brown areas up to 20 mm. in diameter.

EXPERIMENT No. 3 (Apr. 29, 1912).—The tender leaves of two seedlings were inoculated on both surfaces from 5-weeks-old corn-meal-agar cultures (strain 33). These and the two check trees were left under bell jars for six days, the leaves being sprinkled on the second and fourth days. At 10 days infection was just becoming evident, while at the end of one month all but one of the inoculated leaves were peppered with the more or less angular reddish brown spots. After six weeks the development of conidial tufts began to take place on the upper leaf surfaces.

EXPERIMENT No. 4 (May 28, 1912).—The tender leaves of two seedlings were inoculated on the lower surface from a month-old synthetic-agar culture (strain 33) and these, with the single check tree, were covered with bell jars for six days. Observations after three weeks showed the typical spots of this disease up to 3 and in one case 6 mm. in diameter. The conidial tufts were just beginning to form. No infection occurred on the check tree.

EXPERIMENT No. 5 (May 29, 1912).—The young leaves of two seedlings were inoculated from a month-old culture (strain 33) on sterile pecan wood, and the tree was left under a bell jar for several days. At the end of one month numerous somewhat angular reddish brown spots were evident on all the leaves, and these varied in size from mere specks to areas 10 or 15 mm. in diameter. After six weeks the development of conidial tufts had commenced. No infection occurred on the single check tree.

EXPERIMENT No. 6 (June 7, 1912).—The rather mature leaves of two seedlings were inoculated on both surfaces from a 4-weeks-old synthetic-agar culture (strain 113, an isolation from the artificially infected leaves described in experiment No. 1). The air was hot and dry at this time, and hence the bell jars were left over these trees and the three checks for eight days. Observations at the end of two weeks showed the development of typical spots on all the inoculated leaves, and at one month the formation of conidial tufts had begun.

EXPERIMENT No. 7 (3 p. m., May 23, 1912).—The tender leaves of a young tree of the Schley variety at Arlington Farm, Virginia, were inoculated on both surfaces from a 4-weeks-old prune-agar culture (strain 33). The day was cloudy, but the hot, dry weather of the following week prevented infection.

EXPERIMENT No. 8 (2.30 p. m., June 7, 1912).—A second young Schley pecan tree at Arlington Farm was inoculated from a 4-weeks-old synthetic-agar culture (strain 113). The day was cloudy, and the leaves were covered with moistened cotton to further insure the growth of the fungus. The weather was rather hot and dry for several days afterwards, but this period was followed by a day or so of rain. Later observations showed a moderate number of the typical spots on the inoculated leaves, while the check leaves showed no signs of the disease.

EXPERIMENT No. 9 (3 p. m., June 15, 1912).—In a similar manner the four to six young shoots of three Schley pecan trees at Arlington Farm were inoculated from 6-weeks-old corn-meal flask cultures (strain 33). In this case the shoots on one inoculated and one check tree were covered by heavy paraffined paper bags containing moist blotting paper to insure a high humidity around the inoculated leaves, while those on one check and two inoculated trees were left uncovered. Showers occurred on the two following days. Examination in the fall showed many of the typical spots developed on the inoculated leaves covered by the bags and on those of one tree left uncovered. There was no infection on any of the check trees.

EXPERIMENT No. 10 (June 15, 1912).—The young leaves of one potted seedling and the mature leaves of another were inoculated from a 6-weeks-old corn-meal flask

culture (strain 33) and covered with bell jars. An uninoculated check tree was covered in the same way. The trees were sprinkled twice between June 15 and 20, and the bell jars were removed on the latter date, at which time definite infection was noted on the first inoculated tree, but none was on the second or on the check tree. An accident to these trees prevented further observations.

EXPERIMENT NO. 11 (Dec. 18, 1912).—The partly mature leaves of a seedling pecan were inoculated from an 8-weeks-old corn-meal-agar culture (strain 113). This and one check tree were left under a bell jar for three days, when tiny reddish brown specks could be recognized over the inoculated areas. After bleaching and staining, these leaves were examined for the mode of entrance of the fungus into the leaf. Many cases were found in which the mycelial threads had passed through the openings in the stomata. In all probability this mode of infection occurs in the field from the germination of the spores, but this point has not been proved by artificial infection, on account of the lack of distinct conidial formation in culture.

## CULTURAL STUDIES

### THERMAL TESTS

Several series of corn-meal-agar slant cultures were grown for two to three weeks in constant-temperature incubators ranging from 1° to 40° C. No growth took place below 5° or above 35°. After two to three weeks' incubation growth at 8° had barely started, while the rate gradually increased up to 30° (86° F.), this giving the highest rate for the temperatures tested. Growth at 32° was about the same as at 14°. Cultures incubated two to three weeks at 36° and 40° gave no signs of life when subsequently held at room temperature, while those incubated at 2° and 4° made a perfectly normal growth when placed under favorable conditions.

### CULTURAL CHARACTERS

The cultural characters of the fungus as grown upon several of the more common media are briefly described below. No distinct development of conidia has been observed in cultures of the fungus.

Beef-Agar Slant Tubes.—The colonies are convex, approximately raw umber in color, glistening and smooth at first, but later becoming wrinkled and finally attaining a diameter of 10 to 12 mm. Aerial mycelium where present has been very sparse. The submerged mycelium consists of a pale-olive, tangled mass of hyphæ with many swollen and contorted cells.

Beef Broth.—The entirely submerged and dirty-whitish colonies consist of a rounded filmy mass of threadlike mycelium with but few swollen cells. Some of the hyphæ are beaded in appearance.

Corn-Meal-Agar Slant Tubes (Pl. XXXVII, fig. *K*).—The submerged growth which is usually the most prominent part is seal brown to black, while the somewhat cottony aerial mycelium is pinkish. After an incubation of one to two weeks a distinct violet tinge is assumed by the whole agar plug, and the combination of pigment and gelatinous medium gives an opalescent appearance to the whole. Colonies often reach a diameter of 15 mm. Except for the rather scant aerial mycelium, the growth is entirely below the surface of the medium where the hyphæ consist of more or less distorted, dark olive-brown cells.

Corn-Meal Flask Cultures.—The colonies are cottony to plushlike in surface appearance, with a wide variation of color comprising white to pale pink in the cottony parts and shades of raw sienna, burnt umber, and Venetian red elsewhere. A diameter

of 50 or more mm. is often attained by individual colonies after a growth of several weeks.

Filter Paper.—Growth on filter paper moistened with sterile distilled water caused the formation of dark reddish brown circular spots very similar in appearance to those formed on the leaf, while for a radius of 10 to 12 mm. around the spot the paper took on a pinkish cast. An extremely scant white to pinkish aerial mycelium was often developed.

Oxalic-Acid-Agar Slant Tubes.—Colonies are more or less convex, becoming wrinkled with age. The rather scant aerial growth is white to pale pink, while the submerged mycelium is seal brown to black and made up of densely anastomosing and variously contorted hyphæ. The colonies are rarely over 10 mm. in diameter. After several weeks' growth the medium loses its pink color and assumes the shade of ordinary beef agar.

Potato Cylinders.—The colonies are very convex, with white to pinkish aerial mycelium and olive-gray surface growth which becomes much wrinkled with age. A diameter beyond 8 to 10 mm. is rarely attained. The potato cylinder assumes a dark-gray cast for several millimeters beyond the outermost fungous growth, due evidently to enzym action.

Prune-Agar Slant Tubes.—The colonies are little or not at all raised above the surface of the agar, with a fine, velvety, Indian red aerial growth. In the older and drier parts of the culture a scant white to pinkish aerial mycelium develops. A diameter of about 15 mm. is usually attained.

Synthetic-Agar Slant Tubes (Pl. XXXVII, Fig. *J*).—The colonies are extremely convex with a light to dark olive-green velvety surface growth. Numerous guttate drops of liquid are scattered over the surface during the earlier stages. A dark-brown to black, leathery pseudoparenchyma is developed beneath the surface, and with age the whole colony becomes considerably wrinkled. Growth continues until the agar has almost completely dried down, so that the whole slant surface of the medium is often eventually covered by the fungus.

### Morphology and Taxonomy

A comparison of the characters of this fungus with the description of a Clasterosporium published by Heald and Wolf and an examination of their type material deposited in the pathological herbarium of the Bureau of Plant Industry have shown that the two are undoubtedly the same species. Their description is as follows:

*Clasterosporium diffusum.*—Maculis indefinite marginatis, amphigenis; irregularibus, aequaliter brunneis, 5–10 mm. diam.; hyphis effusis prostratis, saepe laxe gregariis atque erectis; conidiis curvulis, clavatis, pluriseptatis, brunneis, 45–135 × 4–5 μ.

On Hicoria pecan (Marsh) Britton. Victoria, 2536; Gonzales, 2695 (type); Yoakum 2770, Halletsville, 2783.

This fungus produces circular or irregular, indefinite margined, brown spots, which are uniformly brown on both surfaces of the leaflets. Dark-brown hyphae run throughout the dead tissue or creep over either surface of the affected area, or are sometimes aggregated to produce clusters of erect conidiophores.[1]

After a careful study of this fungus from both the humid and semi-arid parts of the pecan belt it has seemed to the writer to conform more nearly with the Cercospora than with Clasterosporium characters.

Typically, the latter is saprophytic and possesses a prostrate or creeping mycelium with sporophores either short or differing but little from the

[1] Heald, F. D., and Wolf, F. A. New species of Texas fungi. Mycologia, v. 3, no. 1, p. 21, 1911.

conidia. The latter are borne singly, rarely in clusters, and are largely straight, with rounded ends.

The Cercosporas, on the other hand, are mostly parasitic, and form leaf spots. The sporophores are developed in thick bundles, either through the stomata and from mycelium within the leaf tissues which often takes the form of a stroma beneath the stomatal opening or by sporophores breaking through the epidermis irregularly. The conidia are longish-cylindrical or spindle-shaped, occasionally somewhat club-shaped, straight or bent, and often with a long drawn-out point.

As observed in the humid sections, the typical forms of this fungus had the densely clustered sporophores which, occurring mostly on the upper leaf surface, have arisen from a stroma breaking through the epidermis rather than through the stomatal openings. (Fig. 3.) The mycelium is largely within the leaf tissue and is intercellular, but is also found creeping over the leaf surface and giving rise here and there to single conidiophores. In the semiarid sections the latter type of spore

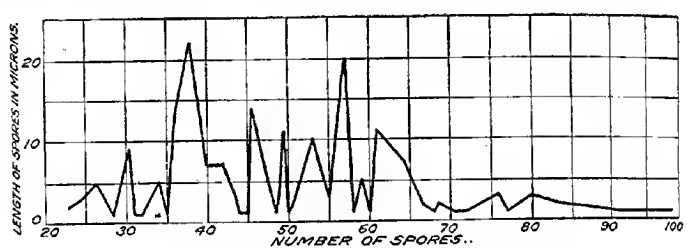



FIG. 4.—Diagram showing measurements in length of 200 conidia.

formation appears to be the more frequent. The conidia are long, usually somewhat club-shaped, and with the apical end the more pointed.

It will be seen that the fungus possesses some characters of both genera. However, since under conditions favorable to fungus growth the Cercospora characters greatly predominate, it has seemed best to place it in this genus. Of course parasitism or nonparasitism should scarcely be given a generic value, but this point at least adds further weight to the present decision. Furthermore, since a *Cercospora diffusa* has been previously described by Ellis and Everhart [1] as occurring upon leaves of *Physalis lanceolata*, it becomes necessary to change also the specific name of this pecan fungus. The emended description of the fungus is given below.

**Cercospora fusca**, emend. sp.

Syn. *Clasterosporium diffusum* Heald and Wolf.

Leaf spots up to 10 or 15 mm. in diameter, at first somewhat angular and bounded by the veins, becoming roundish to irregular and with more indefinite margin, dark reddish brown on both leaf surfaces.

[1] Ellis, J. B., and Everhart, B. M. Additions to Ramularia and Cercospora. Jour. of Mycol., v. 4, no. 1, p. 3. 1888.

Mycelium dark brown and septate, intercellular, sometimes also creeping over the leaf surfaces.

Conidiophores mostly epiphyllous, short and erect, typically in dense, tawny clusters from stromata developed beneath the epidermis and later bursting through, also arising singly from the prostrate surface mycelium.

Conidia pale olive brown, highly variable in size, 30 to 100$\mu$ or more by 3 to 6$\mu$ (see figs. 4 and 5), usually curved, typically subclavate, multicellular, septa less frequent toward the more pointed apical end.

Habitat.—Living leaves of *Carya illinoensis*, Southern States. Also possibly occurring on other species of Carya. Diseased nuts or leaves seen by the writer at Orangeburg, Sumter, and Charleston, S. C.; Americus, Albany, DeWitt, Hardaway, Baconton, Thomasville, Cairo, Bainbridge, and Valdosta, Ga.; Tallahassee, Newport, Monticello, Glen St. Mary, St. Augustine, Palatka, Gainesville, Ocala, and Belleview, Fla.; New Orleans, La.; and at Waring and San Antonio, Tex. Reported also by Heald and Wolf[1] from Victoria, Gonzales, Yoakum, and Hallettsville, Tex.

## PECAN ANTHRACNOSE

[Caused by *Glomerella cingulata* (Stonem.) S. and v. S.]

### HISTORY AND DISTRIBUTION

Pecan anthracnose, variously known among pecan growers as "leaf-blotch" and "rust," was first noted by the writer during the summer and fall of 1910, at which time single-spore strains of the causal fungus were obtained from perithecia matured on the leaves in a damp chamber. Studies of these cultures were carried out during the following winter, and a preliminary description of the fungus later appeared under the name of *Mycosphaerella convexula*.[2]

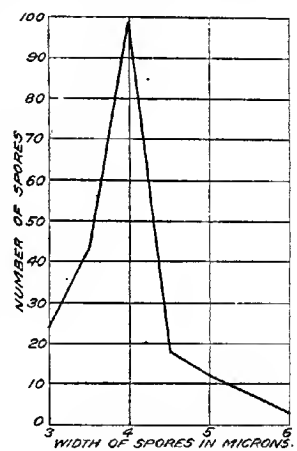



FIG. 5.—Diagram showing measurements in width of 200 conidia.

Further cultural studies of the fungus brought out changes in its morphology sufficient to throw it out of the genus Mycosphaerella, and these, together with cross-inoculation experiments upon the apple, indicated its close affinity to the apple bitter-rot caused by *Glomerella cingulata*.[3] No other published information concerning this disease has come to the notice of the writer.

Pecan anthracnose seems to be well distributed throughout the eastern part of the pecan-growing territory, but it has thus far usually occurred only to a limited extent at any one place. Diseased leaves or nuts have been collected by the writer at Orangeburg, Sumter, Summerton, Charleston, and Aiken, S. C.; at numerous places in southern Georgia and

[1] Heald, F. D., and Wolf, F. A. Loc. cit.
[2] Rand, F. V. A pecan leaf-blotch. Phytopathology, v. 1, no. 4, p. 133–135, 3 fig., 1911.
[3] Rand, F. V. Further studies on the pecan "rust." Science, n. s., v. 35, no. 913, p. 1004, 1912.

northern Florida, including Albany, Dewitt, Baconton, Thomasville, Cairo, and Bainbridge, Ga., and Tallahassee, Newport, Monticello, Jacksonville, St. Augustine, and Belleview, Fla.; and at San Saba, Tex.

## SYMPTOMS OF THE DISEASE

The disease has been found to occur on both leaves and nuts of the pecan. On the leaves it appears in the form of irregular, reddish to grayish brown blotches varying greatly in size and eventually often covering the whole leaf. (Pl. XXXVII, fig. *B*.) The color of the affected areas is the same on both surfaces. Under conditions of moderate humidity, spores of the Gloeosporium type are developed singly, but with favorable temperature and moisture the acervuli with exuding pink spore masses and the black perithecia appear rather thickly scattered over the diseased blotches. When the greater part of the leaf blade becomes involved, it usually falls to the ground, and it is here, under natural conditions, that the acervuli and perithecia are developed.

The blotches on the nuts are also irregular in outline, but are nearly or quite black and often slightly sunken below the surrounding healthy tissue. (Pl. XXXVII, fig. *F*.) The perithecia and the densely gregarious acervuli are formed under the same conditions as on the leaves, but the perithecia have been found on the nuts with much less frequency. A serious dropping of the partially grown nuts sometimes occurs from this cause.

A watery condition of the kernel is frequently found in connection with the anthracnose blotches. It is doubtful, however, whether it has anything to do with this disease, for the same condition prevails both with and without external signs of injury, while both cultural methods and microscopical study have thus far failed to locate any microorganisms in the watery kernels.

## MYCOLOGICAL AND PATHOLOGICAL STUDIES

### ISOLATION OF THE FUNGUS

No mature perithecia have as yet been found on fresh leaves or nuts, but at various times during the last three seasons ripe asci have been readily developed on affected leaves after an incubation of one or two weeks in a damp chamber. The original strains of the fungus were obtained in this way from nursery-tree leaves collected in the fall of 1910 at Baconton, Ga., and were each started from a single, apparently 2-celled ascospore the germination of which was closely followed under the microscope to preclude the possibility of contamination. On several different culture media the colonies at once developed perithecia suggesting the genus Mycosphaerella, and the great majority of the slightly curved ascospores were apparently 2-celled, though a few showed no signs of a cross-septum.

After carrying in culture for about two months, a few spores were noted which were 1-celled, oblong, and slightly smaller than the typical ascospores. These conidia as first noted were borne hyphomycetously, but later were found developing from dense groups of modified fungous cells like an acervulus and in size and shape resembling a typical Gloeosporium. For some time it was thought that this was a contamination, though no possibility of such an occurrence could be found. However, in order to determine this point with certainty, single-spore cultures were started from the 2-celled ascospores and also from the conidia, each individual spore being isolated and its germination carefully followed microscopically. The resulting two series of cultures were similar macroscopically, and both soon developed typical perithecia and ascospores, and also the Gloeosporium conidia. This procedure was repeated 30 or 40 times with a like result in all cases. In several instances the germinating ascospores had within 24 to 48 hours developed mycelial threads which were cutting off conidia in considerable numbers, and in these cases the hyphal connection could frequently be traced from the parent ascospore to the conidium.

However, it was noted after eight or nine months' growth in culture that the 2-celled ascospores were becoming fewer in proportion to the 1-celled, and this tendency has continued until now, after more than two years in culture, the majority of the ascospores are 1-celled, though still of the original form and size.

The possibility suggested itself that perhaps many of the apparent septa were in reality merely a denser layer of cytoplasm across the center of the single cell and that after many generations of growth in culture this cytoplasm had for some reason become more homogeneous. Whatever the explanation, the fact remains that in these original strains a change has taken place from a majority production of apparently 2-celled to that of 1-celled ascospores and that the production of acervuli has become quite as abundant as that of the perithecia. It should be added, however, that many of the ascospores possessed an undoubted septum, as clearly brought out by staining.

During the last two years 10 or 12 other strains of the fungus have been obtained from both conidia and ascospores developed on naturally infected leaves and nuts. In these cases most of the ascospores have been unicellular, though a few have been found with a cross partition clearly brought out by staining.

### Inoculations

Several series of inoculation tests have been carried out on the leaves, but on account of the exigencies of field travel and the difficulty of obtaining suitable material and conditions only two sets of infection experiments have been tried on the nuts.

From the similarity of this fungus to the Glomerella rot of apples and from the omnivorous character of the latter species, as brought out in a paper read by Shear[1] at the 1911 meeting of the American Association for the Advancement of Science, it was decided to make several cross-inoculation tests on the apple. The results of inoculation tests on leaves and nuts and of the cross-inoculation work on the apple are given in the following pages.

LEAVES

EXPERIMENT No. 1 (Feb. 16, 1911).—A distilled water suspension of ascospores from a month-old corn-meal flask culture (strain 17, Baconton, Ga., 1910) was sprayed upon the lower surfaces of six potted pecan seedlings. Three of the seedlings were under bell jars for four days, while the remaining three were left uncovered throughout the experiment. Observations at the end of a week showed no signs of infection, but at four weeks numerous small discolored areas had developed on the foliage of the first three trees and on that of all but one of the others. The three check trees which had been sprayed with distilled water and left under bell jars for four days were sound. No further development of the disease was apparent for some time, but during the latter part of June large, dull reddish brown areas were noted on the leaves of the first three inoculated trees and on one of those which had not been covered with a bell jar. Specimens of these diseased leaves were at once collected, and a microscopical examination showed the development of an occasional Gloeosporium conidium. The leaves were then placed in a damp chamber, and after several days numerous acervuli had developed and were exuding the typical pink spore masses in abundance. Reisolations of the fungus were made from these acervuli.

EXPERIMENT No. 2 (Mar. 15, 1912).—Conidia (strain 17) from 2-weeks-old corn-meal-agar cultures were mixed with sterile distilled water and sprayed upon the leaves of four potted seedling trees, which were then left under bell jars for five days. Two check trees were sprayed with sterile distilled water alone, one being left under a bell jar for five days and the other uncovered. After four weeks it was noted that discolored areas similar to those noted in inoculation experiment No. 1 had suddenly developed, but observation at ten weeks showed no further progress of the disease. The last of May, however, when the leaves were getting well matured, the large, dead, brownish areas were fairly numerous on the leaves of three out of the four inoculated trees. The check trees which had been kept on the same bench but somewhat removed from the infected trees were entirely normal. Specimens of the infected leaves were placed in a damp chamber, where in a few days the Gloeosporium acervuli were formed.

EXPERIMENT No. 3 (Apr. 15, 1912).—Conidial masses from a young corn-meal-agar culture (strain 17) were smeared upon both surfaces of the moistened leaflets of two potted seedlings, one of which was left under a bell jar for several days. Two check trees were similarly treated, but not inoculated. No discoloration of the leaves followed for several weeks, but on May 20 several dead, brownish areas were noted on the leaves of the inoculated tree which had been under a bell jar. These leaves were placed in a damp chamber and in a week had formed numerous acervuli with the typical pink spore masses.

EXPERIMENT No. 4 (May 1, 1912).—Two of the younger leaves from a Sovereign pecan were placed in a damp chamber and sprayed with a sterile distilled-water suspension of conidia from a 2-months-old corn-meal-agar culture (strain 17). The surface of some of the leaflets was slightly abraded with a needle before inoculation. At the end of two weeks large dull-brown areas had developed on most of the leaflets, both

[1] Shear, C. L. Variation in Glomerella. (Abstract.) Science, n. s., v. 35, no. 891, p. 152, 1912.

where abraded and where the surface was left intact. The largest of these irregular spots covered as much as half the surface of the leaflets, and numerous perithecia were forming, though only a few were mature at this time. When 3 weeks old the spots had increased in area so as to involve most of the tissue, and most of the perithecia were mature, bearing the typical curved ascospores in abundance. No acervuli or scattered conidia were noted.

EXPERIMENT NO. 5 (May 29, 1912).—Twenty young seedling pecan leaves were placed in damp chambers and lightly sprayed with a distilled water suspension of conidia and ascospores from a 5-weeks-old corn-meal-agar culture (strain 17). On the third day small brownish areas had developed here and there over the leaf surfaces. On the eighth day these had nearly covered the leaves, and numerous perithecia, together with an occasional acervulus, had developed in the dead tissue. (Pl. XXXVII, fig. *B*.) These fruiting bodies occurred in greater abundance on the lower side of the leaves, but frequently on both upper and lower surfaces. The incipient perithecia and acervuli developed beneath the epidermis, but later burst through and became partly superficial.

EXPERIMENT NO. 6 (Oct. 22, 1912).—Six vigorous but mature leafy pecan shoots were sprayed with a sterile distilled-water suspension of conidia from strain 123 obtained from diseased nuts, and a similar number with strain 150 obtained from a naturally infected apple. The shoots were cut under water and the lower ends placed in bottles of water under slightly ventilated bell jars. Nine check shoots were treated in the same way, except that they were not inoculated.

At three days many of the inoculated leaves in both sets had begun to show the dead, brownish areas characteristic of the disease. After seven days these areas had in some cases involved nearly the whole leaf, with the development of acervuli in moderate numbers. The check leaves were still green and healthy.

EXPERIMENT NO. 7 (Mar. 25, 1913).—Distilled-water suspensions of conidia from one apple strain and three pecan strains of the fungus were sprayed over young seedling pecan leaves in damp chambers. After three days sample leaves from each set were collected and prepared for microscopical examination. A small percentage of the conidia, varying somewhat with the different strains, had sent out germ tubes. Some of the short hyphæ were terminated by appressoria. In one or two cases the germ tube was traced into the opening of a stoma. This method of infection agrees with that described by Shear [1] for Gloeosporiums on a wide variety of hosts.

After five days several of the leaflets in each set exhibited typical infection areas up to 30 mm. in diameter. However, on account of a field trip, no further observations were made on this experiment.

### NUTS

EXPERIMENT NO. 1 (Oct. 22, 1912).—These inoculations were from strain 123, obtained in October, 1912, from blackened nut shucks sent in from Thomasville, Ga., by Mr. C. A. Reed. Terminal shoots bearing healthy green pecans were kindly furnished by Mr. J. B. Johnson, of Manassas, Va. These shoots were cut under water to prevent clogging of the vascular system, placed with the cut ends in bottles of water, and sprayed with a distilled-water suspension of the conidia from this strain. All were then covered with bell jars ventilated at the base to prevent a too great stagnation of the air, but at the same time to furnish sufficient humidity to insure germination of the spores. The check shoots were treated in the same way, except that they were sprayed with distilled water alone.

Group A consisted of 7 shoots bearing 9 nuts, the hulls of which were punctured with a sterile needle and sprayed with sterile distilled water. Group B consisted of 2

[1] Shear, C. L., and Wood, Anna K. Studies of fungous parasites belonging to the genus Glomerella. U. S. Dept. Agr., Bur. Plant Indus. Bul. 252, 110 p., 18 pl., 4 fig., 1913.

shoots treated in the same way but unpunctured. Groups A and B were held as checks. Group C consisted of 8 shoots bearing 10 nuts the hulls of which were punctured with a sterile needle and sprayed with a sterile distilled-water suspension of the conidia. Group D consisted of 6 shoots bearing 8 pecans which were inoculated like group C, except that the hulls were not punctured. Group E consisted of 10 nuts removed from the shoots, their hulls punctured, and inoculated with a suspension of conidia as in groups C and D, and then placed in damp chambers.

At the end of three days distinct infection had occurred on all the nuts with puncture inoculations, the tissue of the hulls being blackened for a radius of 2 to 5 mm. around the needle punctures. The first checks had the tissue blackened for a radius of about 0.5 mm. around the needle punctures, while the nonpunctured check and inoculated nuts at this time showed no evidence of infection. Many of the leaves on the inoculated shoots were developing small, irregular brownish areas, while the uninoculated leaves were all green and healthy.

After nine days groups A and B appeared as on the third day. The very narrow margin of blackened tissue in the punctured checks was due merely to the mechanical injury to immediately surrounding cells, and no further injury occurred throughout the experiment. (Pl. XXXIII, fig. 1, *A*.) All the pecans in group C (Pl. XXXIII, fig. 1, *C*) were blackened over half to the whole of their surface, and acervuli were beginning to develop over the dead parts, with an occasional exudation of the pink spore masses. In group D half of the eight nuts had blackened, and acervuli had begun to develop, but the others gave no evidence of infection. (Pl. XXXIII, fig. 1, *B*.) In group E all the nuts were blackened, and very numerous acervuli with exuding spore masses had developed. Reisolations of this fungus were made as strain 144. Plate XXXIII, figure 2, shows three of the inoculated nuts after further development of acervuli.

APPLES

EXPERIMENT No. 1 (Dec. 30, 1911).—Five sound Jonathan apples direct from cold storage were placed in damp chambers and inoculated by needle punctures, two of them with conidia and three with ascospores inserted directly into the punctures. Three sound apples were punctured with sterile needles and also placed in damp chambers. All were kept overnight at 35° C., and subsequently throughout the experiment at laboratory temperatures. Examination after seven days showed a decay very similar in appearance to bitter-rot around all the inoculation punctures. The check apples were perfectly sound. These cultures were kept for 10 days, with a gradual increase in the size of the decayed areas and formation of incipient fruiting bodies but no spore production.

EXPERIMENT No. 2 (Mar. 5, 1912).—Twelve Yellow Newtown apples were similarly inoculated and placed in damp chambers, one half being inoculated with conidia and the other half with ascospores from an 8-weeks-old corn-meal-agar culture (strain 17). Three apples from each set were held at 28° to 30° C. and three from each set at laboratory temperature. Six apples punctured with sterile needles and placed in damp chambers were held as checks, one half at 28° to 30° and the other half at laboratory temperature. At the end of a week the cultures were examined, and all the inoculated apples had developed a decay apparently identical with bitter-rot, but the brownish and somewhat sunken spots had increased in size much more rapidly at the higher temperature. Two weeks later perithecia were forming and the conidia were developing in considerable numbers, but not in such amount as to give the pink spore masses characteristic of bitter-rot. The check apples at both temperatures remained sound to the end of the experiment.

EXPERIMENT No. 3 (Nov. 30, 1912).—Sound Jonathan and Yellow Newtown apples direct from cold storage were inoculated with three strains of Glomerella obtained from the pecan and with one strain obtained from the apple. The cultures used

were all young corn-meal-agar-slant tubes of the same age and bearing the Gloeosporium stage in abundance. Inoculations were by needle puncture in damp chambers, and, with the exception of group A, all were held at 28° to 30° C. for 48 hours; after this they were kept at laboratory temperature. Group A was held at laboratory temperature throughout.

Group A consisted of three Jonathan apples which were inoculated with strain 17, isolated from diseased pecan leaves collected at Baconton, Ga., in the fall of 1910.

Group B consisted of three Jonathan and four Yellow Newtown apples inoculated with strain 123, isolated in October, 1912, from diseased nuts from Thomasville, Ga.

Group C consisted of four Yellow Newtown apples inoculated with strain 125 similarly obtained from diseased nuts collected at Sumter, S. C., in October, 1912.

Group D consisted of three Yellow Newtown apples inoculated with strain 150, obtained in October, 1912, from an apple naturally affected with bitter-rot.

Group E consisted of six Jonathan and four Yellow Newtown apples treated similarly but not inoculated.

On the fouth day typical bitter-rot areas had developed in all the inoculated cultures. In group A the spots were 1 to 3 mm., while in B to D they were 3 to 20 mm. in diameter. It should be stated that the progress of the tissue decay was somewhat more rapid with strains 125 and 123 than with 150, obtained from the apple itself. In all cases the pale pinkish white mycelium could be seen protruding in tufts from the needle punctures, and the dark-colored fruiting bodies were developing. There were conidia evident at this time. The check apples remained sound. (Pl. XXXIV, fig. A.)

On the eleventh day the spots had increased considerably in size, many of them being 15 to 20 mm. in diameter and becoming confluent. (Pl. XXXIV.) Acervuli extruding the pink spore masses occurred in dense aggregations over the infected tissues, being considerably more abundant, however, in strains 123 and 150 than in the other two. No perithecia had developed as yet, and even after six weeks none had appeared except on the apples inoculated with strain 123.

EXPERIMENT No. 4 (Mar. 21, 1913.)—Sound Yellow Newtown apples direct from cold storage were inoculated as in experiment 3 with two strains of the fungus obtained from diseased nuts, one each from the pecan leaf and the apple, and one originally from the nut but reisolated from an artificially inoculated apple.

On the fourth day bitter-rot areas had developed about the needle punctures in the case of every strain tested, while the check apples remained perfectly sound. (Pl. XXXV.) The decaying spots rapidly increased in size, and after eight days the formation of acervuli had begun.

From these inoculation tests it would appear that this fungus is parasitic on the leaves of the pecan, though usually not actively injurious until a certain stage of maturity of the leaves is reached, together with favorable conditions of temperature and humidity. Field observations also bear out this point.

The limited inoculation work with the nuts, taken alone, would hardly justify very definite conclusions, but as far as they go the experience with leaf inoculations is duplicated. No artificial infection tests have been made upon very young nut hulls, but from the field observations of the last two seasons no evidence of injury during the early part of the season has been obtained. The disease has come to notice only from mid-season on until fall. These observations are in line with the seasonal distribution of bitter-rot as it occurs on the apple.

The cross-inoculations upon the apple, carried simultaneously with infections by the apple bitter-rot fungus, show that the pecan fungus from both leaves and nuts is at least physiologically similar to the Glomerella of the apple. The morphological characters will be discussed later.

## Cultural Studies

### Thermal tests

Several series of corn-meal-agar cultures were grown for two to three weeks at temperatures ranging from 1° to 35° C. As a result of these studies it was found that no growth would take place below 6°, either with freshly inoculated cultures or with those in which growth had already started before incubation. At 7° to 8° the growth was extremely slow, but gradually increased with rise of temperature until the maximum for the strains tested was reached at about 30°.

### Cultural characters

Since the fall of 1910 various strains of the fungus have been grown on the common culture media, and their appearance under different conditions is briefly given as follows:

Beef-Agar Slant Tubes.—The colony first appears as a colorless, roundish, submerged mycelial mass which at ordinary temperatures generally covers the slant in five to seven days, while one to several groups of acervuli or black perithecia have in the meantime usually begun to form. The growth is at first entirely submerged and the surface of the slant presents a smooth glistening appearance. However, after something like two weeks a small amount of whitish aerial mycelium makes its appearance toward the upper edge of the slant. In old cultures this subicle may sparsely cover the whole surface, while the submerged parts become very dark colored.

Corn-Meal Flasks.—Growth becomes visible in two to three days as a roundish colony several millimeters in diameter, with sparse, white to pinkish, cottony aerial mycelium in which are usually scattered a considerable number of dark olive-brown dots. These dots are found to consist of numerous interwoven hyphæ with swollen and contorted cells in process of uniting to form a pseudoparenchyma. These dark masses later develop either into acervuli or perithecia.

Corn-Meal-Agar Slant Tubes.—The white to colorless growth is at first submerged or at the surface. After several days acervuli or perithecia usually begin to form and a scant whitish aerial mycelium may appear at the edges of the slant. The pink spore masses are often developed without the formation of a dark-colored stroma, while in other cases this stroma may be the most conspicuous part of the acervulus. The perithecia are developed within black carbonaceous masses of mycelium which may or may not be submerged in the medium. In old cultures parts of the submerged growth often become olive green to almost black.

Cooked-Potato Cylinders.—Growth first becomes evident through a light-brown discoloration of the tissue immediately around the point of inoculation, and usually a whitish aerial tuft of mycelium appears within 24 hours at the center of the discolored area. This breaking down of the tissue progresses rapidly so that after several days the whole cylinder becomes involved. The white to pinkish cottony subicle develops somewhat more slowly, but eventually covers the cylinder and bears the embedded acervuli or perithecia.

PEDIGREED CULTURES

Starting with a single ascospore and a single conidium from the same strain of the fungus, two series of corn-meal-agar cultures were carried for five generations. Each generation was grown for three weeks before transfers were made for the next succeeding generation, and conditions of temperature and medium were made as uniform as possible throughout the 15 weeks of the test. Observations in every case were taken at three weeks. In the first strain ascospores were always used in making the transfers, while conidia alone were transferred in the second strain.

Ascospore Strain.—Generation 1 had numerous black, carbonaceous, perithecial groups and no acervuli, though a moderate number of conidia were developed hyphomycetously.

In generation 2 the perithecia and acervuli occurred in about equal numbers. In many cases the black perithecial clusters were surrounded with acervuli which were exuding pink masses of spores.

Generation 3 exhibited dense black masses of perithecia near the base of the slants and a considerable number of acervuli which were mostly toward the upper part.

Generations 4 and 5 were similar to the last, except that the two forms were more uniformly scattered over the surface of the cultures.

Conidial Strain.—Generation 1 had numerous acervuli with exuding pink spore masses, but no perithecia.

Generations 2 and 3 had numerous perithecial groups and acervuli well scattered over the cultures, with neither form greatly in predominance.

Generation 4 had numerous pink spore masses along the streak, and perithecial clusters in moderate numbers near the base of the slant.

Generation 5 had both forms in about equal numbers and well scattered over the surface of the cultures.

Further cultural studies carried out in the same way as the one described above have given essentially the same results—namely, that a strain producing both spore forms will continue to produce both ascospores and conidia even though one form alone is used in reproduction. Variations have occurred from time to time, but these have occurred irregularly and without continuance. Strains of the fungus from single ascospores have sooner or later always given rise to both ascigerous and conidial forms. However, some conidial strains have been obtained from the host which, after two years in culture, still produce only the conidial form. It would thus appear that there are conidial strains of the fungus which have lost the power of developing the perfect stage or which at least have not met with the proper inciting conditions.

MORPHOLOGY AND TAXONOMY

The perfect stage has been noted less frequently than the conidial stage, but nevertheless the perithecia have been occasionally found on both leaves and nuts. The first evidence of perithecial formation is seen in a plexus of pseudoparenchyma tissue made up of more or less isodiametric fungous cells developed in the decaying tissues beneath the epidermis. This finally develops into the mature perithecium which ruptures the

epidermis and becomes partially superficial. The mature fruiting body is nearly spherical, but is papillate and occasionally short beaked. From several hundred measurements it has been found to vary from 80 to 250$\mu$ in the longest diameter, with the majority lying between 150 and 200$\mu$. The perithecia are black and carbonaceous, and in culture several are usually developed within a single carbonaceous stroma.

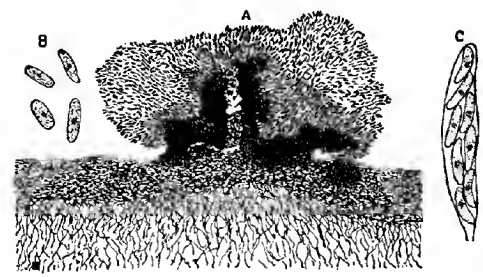



FIG. 6.—The anthracnose fungus upon corn-meal agar. *A*, Acervulus, X84; *B*, conidia, X400; *C*, ascus, X400.

The 8-spored asci vary considerably in size and shape, but are usually cylindrical-clavate. (Fig. 6.) The extreme measurements found were 45 to 80 by 9 to 12.5$\mu$, the majority lying within the limits of 55 to 80 by 10 to 11$\mu$.

The ascospores are unicellular (rarely with a cross partition), oblong, slightly tapering toward both ends, and usually curved. (Fig. 6.) The extreme measurements found were 12.5 to 29 by 3.5 to 6$\mu$, the majority lying about midway between the two extremes as shown in the accompanying graph (fig. 7) drawn from measurements of 150 spores of a single strain taken at random and all developed in corn-meal-agar culture. Measurements of other strains both from culture and from the host have come within these limits.

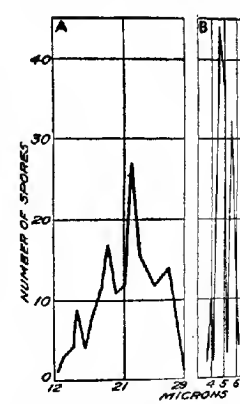



FIG. 7.—Diagram showing ascospore measurements of the anthracnose fungus. *A*, Length of 150 ascospores; *B*, width of 150 ascospores.

The acervuli have been of much more common occurrence on the host. (Fig. 6.) In their early stages they are scarcely to be distinguished from the perithecia, but the production of the characteristic pink spore masses soon differentiates them even macroscopically from the perfect stage. The production of setæ has been found of frequent though by no means of general occurrence, and to vary even within a single strain.

The conidia are ovate to oblong, with blunt, rounded ends (fig. 6) (occasionally somewhat dumbbell-shaped). Both on the host and in culture they have been found to develop hyphomycetously, as well as in acervuli. The measurements taken from several strains on the host

and in culture ranged within the limits of 11 to 22 by 3.8 to 7.6$\mu$. The accompanying graph (fig. 8) shows the measurements of 150 conidia taken from the same strain and under the same conditions as those noted above for the ascospores. The conidia have frequently been found to develop appressoria as described by various authors for the apple bitter-rot fungus.

From the general pathology and temperature relations, the cross-inoculation and cultural studies, and finally from the morphology of the pecan fungus there can be no doubt of its specific connection with *Glomerella cingulata* (Stonem.) S. and v. S., the fungus causing bitter-rot of apple, ripe-rot of grape, and anthracnoses of a wide range of hosts.

In several instances Glomerella perithecia have developed upon pecan leaves scattered among the densely gregarious pycnidia of a fungus which has since proved to be *Phyllosticta convexula* Bubák.[1] The spores of the latter are almost bacillar in size, measuring 1.5 to 2 by 1$\mu$, while in many cases only a few pycnidia upon a leaf matured in damp chamber, so that morphologically most of these fruiting bodies were similar to the immature perithecia of Glomerella.

Furthermore, an examination of the fruiting bodies from type specimens of *Sphaerella convexula* (Schwein.) von Thüm.[2] (*Sphaeria convexula* Schwein.[3]) shows them to be morphologically similar to those of *Phyllosticta convexula*. The original brief diagnosis of *Sphaerella convexula* was from immature material and without description of asci or ascospores. Similar material has been collected by the writer at various points in South Carolina, Georgia, and Florida, including one of the type localities of the species.

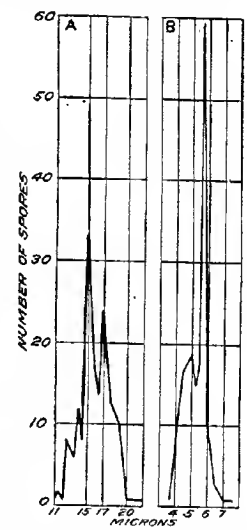



FIG. 8.—Diagram showing conidial measurements of the anthracnose fungus. *A*, Length of 150 conidia. *B*, width of 150 conidia.

Glomerella perithecia have been developed in a damp chamber, not only upon disinfected pecan leaves exhibiting the typical anthracnose blotches and among the pycnidia of *Phyllosticta convexula*, but also frequently upon leaves apparently healthy in every respect, showing the wide distribution of the former fungus and its ability to hibernate on the living host until the occurrence of conditions favorable to its further growth.

[1] Bubák, Franz, Einige neue Pilze aus Nord America. Jour. Mycol., v. 12, no. 82, p. 52, 53. 1906.
[2] Saccardo, P. A. Sylloge Fungorum. v. 1, Patavium, 1882, p. 494.
[3] Schweinitz, L. D. von. Synopsis fungorum in America boreali media degentium. Trans. Amer. Phil. Soc. n. s., v. 4, p. 224, 1834.
Berkeley, M. J. Notices of North American fungi. Grevillea, v. 4, no. 32, p. 154, 1870.

From these facts it seems entirely possible, if not indeed probable, that the type fungus of Schweinitz and Von Thümen was in reality identical with *Phyllosticta convexula* Bubák and that the immature perithecia were those of the fungus at present known as *Glomerella cingulata*.

## KERNEL-SPOT

[Caused by *Coniothyrium caryogenum*, n. sp.]

### HISTORY AND DISTRIBUTION

Fortunately this disease has hitherto been of only occasional occurrence. In the fall of 1907 infected kernels were received by Mr. W. A. Orton, Pathologist in Charge, Cotton and Truck Disease and Sugar-Plant Investigations, Bureau of Plant Industry, from Minden, La., accompanied by the following statement:

> The disease of the pecans is not confined to any one tree or variety. * * * For six years they have contained the blight, growing worse each year, until I think that next year there will not be a single good one (nut) found among them. * * * I have never heard of this disease from anyone else. All our trees are infected.

From these specimens Orton isolated a fungus with brown, septate, branched mycelium. No further studies were carried out to determine its parasitism or further cultural characters,[1] but examination of these specimens has shown them to have the symptoms associated with the kernel-spot.

No other definite reports of the kernel-spot prior to 1910 have come to notice, but during the last three years occasional specimens from a number of Southern States have been received by the Office of Fruit-Disease Investigations. Among these communications the only report of serious injury was from a point in southern Georgia, where in the fall of 1911 most of the nuts on a large seedling tree were rendered unfit for consumption. From this source were obtained the fungous cultures used in the present inoculation work. Since there were no nuts on this tree the following year, field studies as to time and manner of infection could not be carried further.[2]

### SYMPTOMS OF THE DISEASE

Externally there is no evidence of infection and it is only upon freeing the kernel from the shell that the diseased condition becomes apparent. (Pl. XXXVII, fig. *E*.) On the surface of the kernel the spots are dark brown to almost black and often slightly sunken. They are in general irregularly roundish with a fairly definite margin and several millimeters in diameter. Internally the diseased tissue extends in an approximate

[1] Orton, W. A. From unpublished notes.

[2] If a tree is badly infected, the nuts should be gathered and burned, in order to lessen the chances of further spread of the disease.

hemisphere beneath the dark-colored spot. The central part of this hemisphere is dry and pithy, slightly discolored, and surrounded by a definite dark-brown layer separating the diseased from the healthy parts. The tissues are slightly disorganized, but are not softened or entirely broken down. A bitter taste is imparted to the kernel. Microscopically, the fungus is found to enter the cells of the kernel, where the hyphæ become partially broken up into their constituent cells. Outside the dark-colored boundary layer the tissues of the kernel are seen to be slightly discolored, although no signs of the fungus itself are seen here. It seems probable that enzyms or toxins (or both) excreted by the fungus may diffuse out into the healthy cells of the host and by partial digestion prepare the way for the entrance and progress of the parasite.

## MYCOLOGICAL AND PATHOLOGICAL STUDIES

### Isolation of the Fungus

The affected pecan kernels received in the fall of 1911 from Thomasville, Ga., were washed for five minutes in a solution of bichlorid of mercury (1:500), and in distilled water. Small pieces of the diseased internal tissue were then cut out under sterile conditions and transferred to Petri dishes of melted beef agar. Yellowish bacterial colonies resulted from two of the transfers, but a constant fungous type developed from all the others. The bacteria and the fungus were isolated and carried in pure culture for the following cultural and inoculation studies.

### Inoculations

In all the inoculations the kernels were freed from the shells under semisterile conditions and placed upon sterile, moist filter paper in Petri dishes. Under these conditions the pycnospores or mycelium from a pure culture were placed upon the kernels either with or without slight abrasion of the surface. The checks were treated in a similar manner but without inoculation.

Experiment No. 1 (Jan. 15, 1912).—The kernels from several stratified nuts were placed in Petri dishes and inoculated by slight abrasion (1) with spores of the fungus (strain 99), and (2) with the yellow bacteria (strain 101), while the kernels in the third dish (3) were merely abraded with a sterile scalpel. After eight days typical symptoms of the kernel-spot had developed in the first culture. (Pl. XXXVII, fig. *D*.) The bacteria in the second culture had made a slight growth, causing an irregular softening of the superficial tissues, but without discoloration or other resemblance to the kernel-spot. The check cultures were entirely sound.

Experiment No. 2 (Jan. 25, 1912).—Kernels of well-cured Stuart pecans were inoculated with the fungous spores, six kernels upon the uncut surface, and eight with a slight abrasion. Four kernels were held as checks. After 12 days typical spots had formed upon half of the first set and on all of the second set of kernels. Of the checks, two kernels were perfectly sound, the third exhibited a slight bacterial softening at one end, and the last was softened throughout by a growth of *Penicillium*

*glaucum*. In the last two cases the injury was similar in no particular to the kernel-spot.

EXPERIMENT NO. 3 (May 13, 1912).—Three Petri dishes containing four to eight kernels from cured pecans were inoculated by placing macerated pycnidia upon the uninjured surfaces. A fourth Petri dish was held as a check. After seven days it was noted that infection had taken place at every point of inoculation in the first two cultures. In the third, two kernels had become infected with the kernel-spot, but the remaining two were entirely softened by bacterial contamination. In the check Petri dish two kernels were sound and two were contaminated and softened throughout by *Botrytis cinerea*. In no case was the injury by contamination similar to the disease under investigation.

EXPERIMENT NO. 4 (Nov. 20, 1912).—Ten kernels of newly harvested pecans were inoculated with macerated pycnidia and without abrasion of the surface skin. A similar number of kernels were held as checks. After nine days, 8 out of the 10 inoculated kernels had developed the disease. The checks were sound, except for two or three kernels which had softened and yellowed throughout from bacterial contamination.

EXPERIMENT NO. 5 (Dec. 25, 1912).—Eight to ten partially cured kernels of each of the following varieties were inoculated with macerated pycnidia by a slight abrasion of the surface: Schley, Curtis, Nelson, Teche, Alley, Pabst, and Van Deman. Check kernels of each variety were carried throughout the experiment. Similarly, Teche and Van Deman kernels were inoculated with the two strains of yellow bacteria (strains 100 and 104). After five days the bacterial inoculations had caused a softening of the tissues throughout, but there were no evidences of the kernel spot. The fungous inoculations had in nearly every case taken, and spots typical of the disease both externally and internally had developed, regardless of variety. The checks were sound, except for an occasional contamination with *Botrytis cinerea*, which had caused a general softening of the tissue. Reisolations of the fungus were made from each of the varieties inoculated, and one of these strains was used in the next experiment.

EXPERIMENT NO. 6 (Jan. 6, 1913).—Three Petri dishes of partially cured Van Deman kernels were inoculated upon the slightly abraded surface with macerated pycnidia of the fungus reisolated from artificial inoculation in experiment No. 6. Three dishes of kernels were similarly inoculated with a Sphaeropsis obtained from old decaying pecan hulls, while two were held as checks. Observations after five days showed infection with typical symptoms in every case of inoculation with the kernel-spot fungus. The Sphaeropsis had caused a general breakdown and softening of the tissues, with slight discoloration, but with no symptoms like the disease in question. The checks all remained sound and free from infection of any kind.

No opportunity for field inoculations has presented itself without the accompanying danger of introducing or spreading the disease, and hence the infection tests have been entirely confined to the laboratory. However, the characters of the disease are so definite and the results of the inoculation work on kernels in the laboratory have been so largely positive that the fungus tested (strain 99 and its reisolation) may now be legitimately regarded as the cause of the kernel-spot. The general disorganization and moist softening of the tissues brought about by the bacteria and by the Sphaeropsis, Botrytis, and Penicillium fungi was entirely different in appearance and result from the disease under investigation. Individual infections of the latter occur within limited and well-defined boundaries and, though giving a pithy consistency to the diseased parts, never cause a moist softening of the injured tissue.

### Cultural Studies

As grown upon corn-meal agar the optimum temperature for the fungus was found to lie around 20° C. (68° F.). No growth took place below 2° or above 37°. The rate was slow at 4°, but gradually increased up to the optimum, and decreased somewhat more rapidly in rate above that point. At 35° a slight but abnormal growth occurred for a few days, but at the end of the 3-weeks' test, incubation at the optimum temperature failed to show any further signs of life in these cultures.

Upon corn-meal agar the submerged growth varies but little from a sepia brown, while the aerial mycelium shows gradations from that to whitish. Usually a large number of dark-sepia to almost black pycnidia are formed upon this medium. The mycelium is straight and but little branched, with gradations from brown to almost hyaline.

On corn-meal flasks the colonies appear very much as upon the corn-meal agar, though the aerial mycelium is usually much more luxuriant and cottony, becoming, however, somewhat felted with age. Pycnidia are developed in large numbers.

On cooked-potato cylinders the colonies are brown ocher, varying also to a slightly darker shade. The surface is smooth and glistening, becoming somewhat wrinkled with age. No aerial mycelium or pycnidia have been observed on this medium. The cells of the hyphæ differ from those grown upon corn-meal agar in being more nearly isodiametric, with thicker and somewhat bulging walls. The mycelium possesses but few side branches, and the color varies from pale brown to almost hyaline. In cultures several weeks old the whole potato cylinder becomes somewhat softened and turns brown, but no fungous mycelium is found except near the surface. The starchy contents of the potato cells become largely digested, though the walls of the deeper lying cells remain intact except for the breaking down of the middle lamellæ.

Upon synthetic agar the growth is brown ocher to sepia in the older and drier parts. The surface growth often becomes more or less wrinkled and moist-mealy in appearance in older cultures, while a pale brown to whitish aerial mycelium may or may not develop. Microscopically the hyphæ very much resemble those developed upon the potato cylinders, but the thickening and bulging of the walls is often much more apparent. Indeed, the hyphæ frequently break up into their constituent cells, and it is this behavior that gives the moist-mealy appearance to some cultures.

### Morphology and Taxonomy

The study of this fungus in culture and upon the host has shown it to conform in characters with the genus Coniothyrium. However, no member of this genus has been found hitherto reported on the pecan or any nearly related host. It thus becomes necessary to give the fungus a new specific value until cultural and cross-inoculation work can establish

its connection with a previously described Coniothyrium occurring upon some widely differing host. An enumeration of the characters thus far observed is given below.

It should be stated that the pycnidia have been observed mostly in culture, their formation on the host having been confined to the extracted kernels in a damp chamber. In the latter case their development has has taken place at or near the surface of the kernel and often accompanied by a thin subicle of brown to whitish hyphæ.

**Coniothyrium caryogenum, n. sp.**

Upon pecan kernels *Coniothyrium caryogenum* causes dark-brown, irregularly roundish surface spots with a hemisphere of pithy tissue beneath, which is surrounded by a brownish layer of host cells.

Mycelium brown, sometimes almost hyaline where not submerged, septate, slightly branched, straight or within the host cells often separating into the constituent hyphal cells which are then more or less swollen and thick walled.

Pycnidia roundish, osteolate, thin walled, dark brown, about 200 to 250 $\mu$ in diameter.

Sporophores short and indistinct. Spores pale brownish, elliptical, 1-celled, 2.5 to 3.6 by 1.8 to 2 $\mu$.

Habitat.—Kernels of *Carya illinoensis* (Wang.) K. Koch. Type specimens from large seedling tree belonging to Mr. James R. Vann, Thomasville, Ga. Specimens also received from Raleigh, N. C.; Baconton, Ga.; Monticello, Fla.; Minden, La., and other points in the pecan belt, including Texas.

## CROWN-GALL

[Caused by *Bacterium tumefaciens* Sm. and Town.]

So far as known, the crown-gall has not hitherto been published as occurring on the pecan from natural infection. However, in the fall of 1909 specimens of young trees affected with both the hard and soft types of galls (Pl. XXXVI) were received from a nursery in Mississippi with the statement that about 0.1 per cent of the stock in the nursery was infected. The disease has also been observed by the writer at one point in northern Florida. But, since these two localities have furnished the only cases reported, it may be considered as of very rare occurrence upon this host.[1]

On the pecan the tumors occur not only at the collar of the tree but several inches higher up on the stem and also on the roots. The greater prevalence of the disease near the surface of the ground is explained by the fact that the parasite first enters the host tissues through wounds. Thus, the process of grafting and the subsequent treatment of the stock readily furnish conditions requisite for infection and further development. The typical appearance of the disease may be inferred from the name; the galls at first consist of a succulent growth of the young host cells thrust out from the cambium layer in the form of a tumor which may attain a considerable size. With age the surface becomes much

[1] The only practical method of control hitherto employed consists in rigid nursery inspection. Obviously, no trees showing the disease should be planted, even though the pecan does not appear to be as seriously affected as many other plants.

roughened and darker in color and the interior tissues are then more or less distorted and hardened. Often the interior assumes a distinctly woody texture, and a roughened bark develops over the surface to form the "hard-gall" type. With the development of roots from the tumor tissue the "hairy-root" type appears, but this form has not been observed on the pecan.

### EXPERIMENTS WITH THE CROWN-GALL ORGANISM

Soft galls from the Mississippi nursery (December, 1909) were left for five minutes in a solution of corrosive sublimate (1:500) and washed in sterile distilled water. Small pieces of the abnormal tissue were then removed under aseptic conditions from points just under the surface and near the edge of the galls, and beef-agar cultures started by the ordinary poured-plate method. In from three to eight days the circular and somewhat opalescent colonies of the organism appeared, but were much more abundant in cultures started from the extreme base of the young soft galls near the juncture between the diseased and healthy tissues. Transfers were made to beef-agar slant tubes, and with one of the strains thus obtained the following inoculation tests were made.

EXPERIMENT No. 1 (December, 1910).—Six table beets were inoculated by needle punctures from young beef-agar cultures of the bacteria, while a like number of beets were punctured with sterile needles and held as checks.

After five weeks, examination of the inoculated beets showed the development of typical galls, 3 to 10 mm. in diameter, at most of the needle punctures, while the checks showed no signs of infection.

EXPERIMENT No. 2 (Jan. 12, 1911).—Four potted pecan seedlings were inoculated by scalpel punctures at the crown from 4-day-old beef-agar cultures, and the soil was replaced around the base of the tree to preserve the moist condition. Four other seedlings were treated in the same manner, except that no bacteria were introduced. The trees were all dormant at this time and remained in this condition until the latter part of March, when, with the exception of one of the inoculated trees which died from other causes, all pushed out their foliage in the normal manner.

Examination in June showed a tumor several millimeters in diameter at the crown of one of the inoculated trees and an apparently incipient infection on a second. All the other trees had completely healed over, so that the location of the punctures could scarcely be made out. On September 12, eight months after inoculation, well-developed galls were found at the crown of two out of the three remaining inoculated trees. The check trees, together with 59 other pecan seedlings in the same greenhouse, showed no indications of the disease.

Since these brief studies with the parasitic organism were carried out merely to indicate the connection of this disease of the pecan with the well-known crown-gall, no further inoculation and cultural tests were made. However, cultures of the bacterium were submitted to Dr. Erwin F. Smith, of the Bureau of Plant Industry, who obtained similar results in inoculation experiments and further verified the identity of the organism with *Bacterium tumefaciens* Sm. and Town., the cause of crown-gall of plants.

SUMMARY

The nursery-blight is a serious disease of young trees, but is rarely found to be injurious in orchards. Its distribution corresponds closely with that of the host. The casual fungus, *Phyllosticta caryae* Peck, attacks only the leaves of the pecan. Infection first becomes evident through the formation of tiny circular, dark-brown spots, which increase gradually in size and finally become grayish white in the center of the upper surface and usually blackish throughout on the lower. Entire defoliation of young trees sometimes takes place. Spraying with Bordeaux mixture has proved a very effective method of control. Since the disease is primarily a nursery trouble, the question of disease resistance would not be applicable in this connection. All attempts at pure-culture inoculation have been successful. A combination of high humidity and temperature seem best to favor the spread of the disease. The fungous mycelium ramifies through the intercellular spaces above the lower epidermis and throughout the mesophyll tissue. Pycnidia are few on the living leaves, but are produced in abundance on some culture media.

The brown leaf-spot usually causes very little injury, but is widely distributed and occasionally during wet seasons some defoliation may result. The fungus *Cercospora fusca*, emend. sp., causes dark reddish brown spots of uniform color on both leaf surfaces. These are at first somewhat angular in outline as bounded by the veins of the leaf, but may later become roundish and more indefinite in their margins. There appears to be little difference in resistance to this disease among the varieties now commonly planted. The rather limited observations upon the effect of Bordeaux mixture were favorable to the control of the disease. Pure-culture inoculations were highly successful, giving the typical disease symptoms. The temperature relations were very similar to those of the nursery-blight. The mycelium is largely intercellular in its growth, but aggregations of fungous cells break through the upper epidermis to bear the pale tawny conidial clusters, and a creeping surface mycelium sometimes occurs. True spore formation has not taken place in culture.

The pecan anthracnose is well distributed, but hitherto has not usually been very serious at any one point. It has been shown by cultural and cross-inoculation work to be due to *Glomerella cingulata* (Stonem.) S. and v. S., the fungus causing bitter-rot in apples. On the leaves infection causes the formation of irregular reddish to grayish brown blotches varying greatly in size and eventually often covering the whole leaf. On the nuts the blotches are also irregular in outline, but nearly or quite black and often slightly sunken below the surrounding healthy tissue. The production of acervuli and perithecia occurs under suitable conditions of temperature and humidity. The problem of control is largely in the tentative stage, though from the work of Scott and others

on the apple bitter-rot it is thought that Bordeaux mixture will prove effective. Some indications of difference in varietal resistance have been observed. High temperature and humidity furnish the optimum conditions for growth and spread of the disease, as is the case with the bitter-rot of apple.

The kernel-spot is fortunately rare, but on this account the present study has been largely confined to laboratory and greenhouse work. The fungus *Coniothyrium caryogenum*, n. sp., causes the development of dark brown to almost black surface spots upon the kernel. Internally the diseased tissue extends in an approximate hemisphere beneath the dark-colored spot and is pithy in texture and bitter to the taste. Pure-culture inoculations have been largely successful. The optimum temperature for growth was found to be about 70° F. The mycelium enters the cells of the kernel, where it is often more or less swollen and broken up into its constituent cells. Pycnidia have been produced abundantly in culture, but on the host only on the extracted kernels in a damp chamber.

Crown-gall has been found on the pecan in northern Florida and southern Mississippi. It is similar in appearance to the well-known crown-gall of plants and has been shown by pure-culture and inoculation work to be due to the same organism, *Bacterium tumefaciens* Sm. and Town.

## DESCRIPTION OF PLATES

PLATE XXXIII. Fig. 1.—Pecan nuts infected with the anthracnose fungus by spraying with a distilled water suspension of conidia, showing the appearance nine days after inoculation. Natural size. Fig. *A*.—Four check nuts, two punctured with sterile needle and two unpunctured. Fig. *B*.—Four nuts inoculated upon the unpunctured surface of the hull. Fig. *C*.—Four nuts inoculated after puncturing the surface of the hull with a sterile needle.

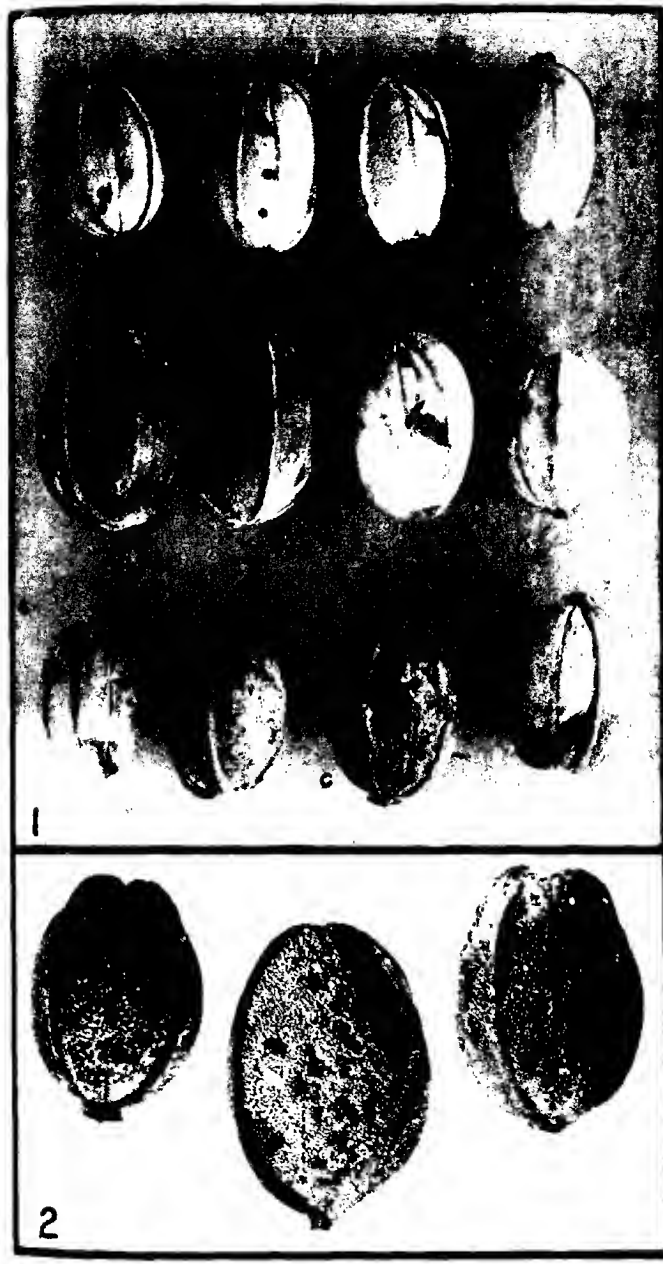
Fig. 2.—Three of the infected nuts shown in figure 1 after further development of the acervuli. × 1½.

XXXIV. Yellow Newtown apples infected by needle puncture with conidia of the anthracnose fungus from pecan and apple, showing appearance four days after inoculation. One-half natural size. Fig. *A*.—Check apples punctured by sterile needle. Fig. *B*.—Apples infected by needle punctures with strain 150 from the apple. Fig. *C*.—Apples infected with strain 123 from a diseased pecan hull. Fig. *D*.—Apples infected with strain 125 from a diseased pecan hull.

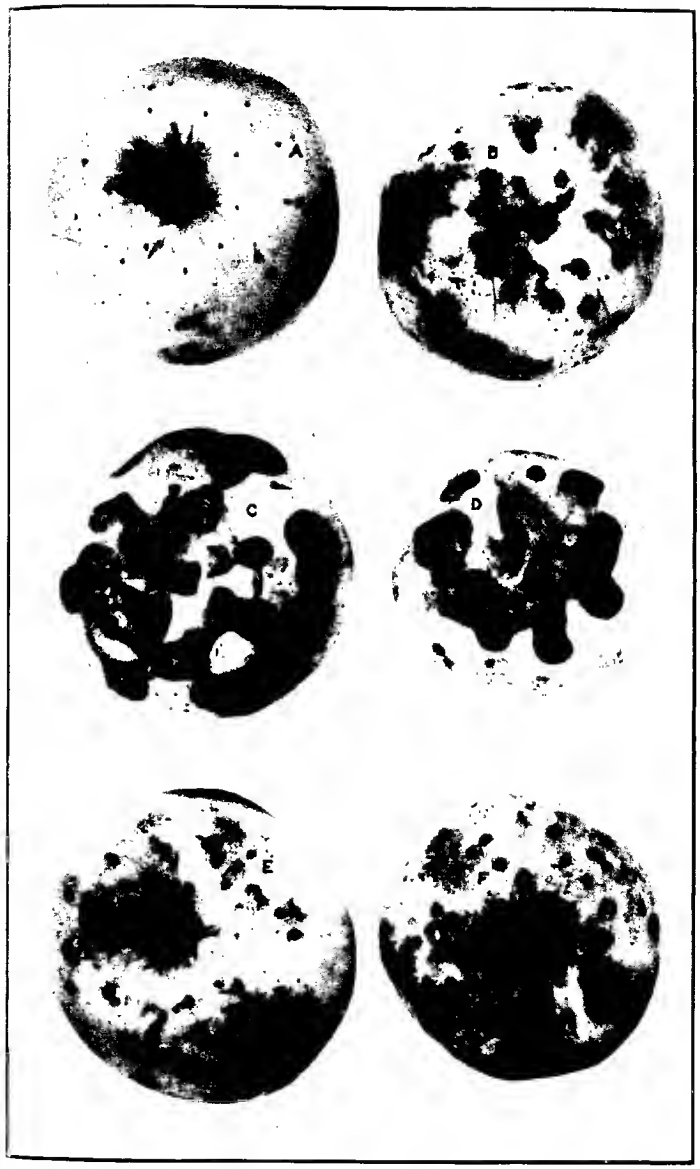
XXXV. Yellow Newtown apples infected by needle puncture with conidia of the anthracnose fungus from pecan and apple, showing appearance four days after inoculation. Two-thirds natural size. Fig. *A*.—Check apple punctured by sterile needle. Fig. *B*.—Apple infected with strain 125 from the pecan nut. Fig. *C*.—Apple infected with strain 123 from the pecan nut. Fig. *D*.—Apple infected with strain 150 from the apple. Fig. *E*.—Apple infected with strain 146 from the pecan leaf. Fig. *F*.—Apple infected with strain 158, a reisolation of strain 125 after passage through the apple.

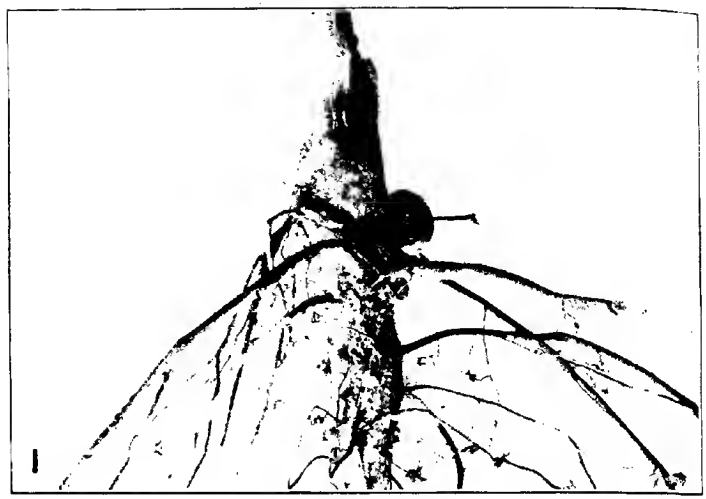
XXXVI. Crown-gall (caused by *Bacterium tumefaciens* Sm. and Town.) on pecan nursery trees from southern Mississippi. Natural infection. Two-thirds natural size. Fig. 1.—The soft type of gall. Fig. 2.—The hard type of gall.

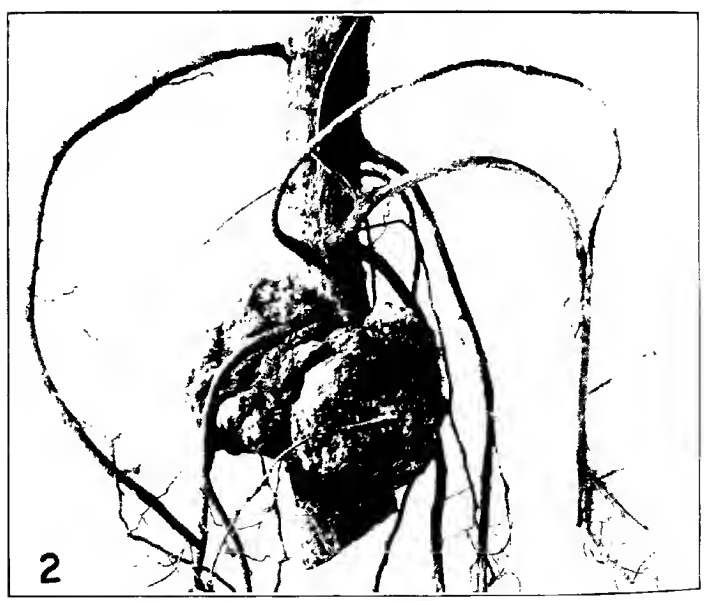
XXXVII (colored). Fig. *A*.—A pecan leaflet infected with the brown leaf-spot fungus (*Cercospora fusca*, emend. sp.) from pure culture. Fig. *B*.—A pecan leaflet infected with the anthracnose fungus (*Glomerella cingulata* (Stonem.) S. and v. S.) from pure culture. Fig. *C*.—View of upper surface of a pecan leaflet recently infected with the nursery-blight fungus (*Phyllosticta caryae* Peck) from pure culture. Fig. *D*.—A pecan kernel infected with the kernel-spot fungus (*Coniothyrium caryogenum*, n. sp.) from a pure culture, showing the appearance eight days after inoculation. Fig. *E*.—A pecan kernel with the kernel-spot from natural infection. Fig. *F*.—A pecan nut infected with the anthracnose fungus from pure culture. Fig. *G*.—The nursery-blight fungus upon synthetic agar after two weeks. Fig. *H*.—The nursery-blight fungus on corn-meal agar after two weeks. Fig. *I*.—Views of the upper and lower surfaces of pecan leaflets, showing an advanced stage of the nursery-blight. Natural infection. Fig. *J*.—The brown leaf-spot fungus on synthetic agar after four weeks. Fig. *K*.—The brown leaf-spot fungus on corn-meal agar after four weeks. (All figures are natural size.)


1
2


A
A
B
B
C
C
D
D


A
B
C
D
E


1


2


A
B
C
D
E
F
G
H
I
J
K

# A TWIG BLIGHT OF QUERCUS PRINUS AND RELATED SPECIES

By DELLA E. INGRAM,
*Scientific Assistant, Investigations in Forest Pathology, Bureau of Plant Industry*

## INTRODUCTION

A twig blight of the chestnut oak (*Quercus prinus* L.) was first reported to the Office of Investigations in Forest Pathology on May 31, 1911, by Drs. Metcalf and Spaulding, of that office. Specimens were collected and sent in from York, Pa. Since that time the disease has been reported and diseased specimens have been received from various points throughout Virginia, West Virginia, Maryland, Pennsylvania, New York, and Connecticut. It is not possible at this time to determine definitely the exact range of the blight, as sufficient data have not been obtained. Nothing is known regarding the origin, age, or directions of distribution of the causal fungus, but apparently it will seriously lower the silvicultural status of the chestnut oak.[1]

## EFFECT ON HOST

This blight is primarily a disease of the chestnut oak, but occasionally the American chestnut (*Castanea dentata* (Marsh) Borkh.) and the white oak (*Quercus alba* L.) are attacked. Inoculations in the greenhouse have proved that a number of other species of oak are also susceptible.

Trees of all ages and sizes may be attacked, but usually only the small branches of the larger trees are affected. In some cases where young saplings are attacked the whole tree is killed outright. On the affected twigs the leaves wither suddenly without yellowing, gradually shrivel, and turn a chocolate brown. This browning of the leaves and twigs gives the tree the appearance of the well-known fire-blight of the pear and the apple. (Pl. XXXVIII.) The fungus often stops at the point where the secondary shoots join the main stem, and, as a result, the affected twig may rot at the base and fall off. On the diseased twigs are numbers of small black pycnidia erumpent through the bark. These are sometimes arranged singly and sometimes grouped. Careful sections were made of leaves from diseased twigs brought in from the field, but no mycelium could be found in the tissues. Cultures were also made, but nothing developed. A microscopic examination of a transverse section of the wood reveals the presence of abundant mycelium in the

[1] Apparently the only practical method of control for individual trees is cutting back the young twigs several inches below the darkened portion. However, under forest conditions no practicable means of control is known.



17073°—14——5

tracheary tubes and throughout the cells of the inner and outer bark. A study of the distribution of the mycelium in the twigs of different ages and the relative amount present in the wood and cambium of the diseased twigs was not undertaken.

## MORPHOLOGY OF FUNGUS

Soon after the leaves wither on the affected twigs, small papillæ begin to form under the bark, which in the course of a few weeks break through

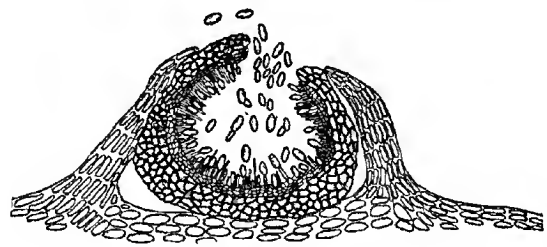


FIG. 1.—*Diplodia longispora:* A section of a pycnidium.

in the form of the small, black pycnidia mentioned above. These are globose to subglobose in shape, very distinctly ostiolate, and dark brown to black in color. In size they vary from 95 to 145$\mu$ in diameter. In cross section (fig. 1) the wall of the pycnidium is made up of practically two parts: The outer, dark carbonlike layer and an inner membranous layer of typical fungous cells. These cells have a decidedly purplish tinge, merging into hyaline as the sporogenous layer is reached.

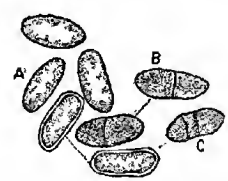



FIG. 2.—*Diplodia longispora:* Stages in development of spore. *A*, Macrophoma stage; *B*, Diplodia stage; *C*, Diplodia spore with two septa.

The spores both on the host and in culture are oval or ovoid (fig. 2, *A*), often tapering somewhat at one end, densely granular, often very thick-walled, averaging about 29 × 11$\mu$ in size. At first the spores are hyaline and continuous, but after some time (fig. 2, *B*) they take on a yellowish tinge and finally become dark brown in color and 1-septate. Rarely the septum forms in the hyaline spores before the color begins to change, but this is not usually the case.

The spores are borne singly on rather short, broad conidiophores, interspersed with numerous filiform paraphyses, and are abjointed from the tip at maturity by a constriction near the end of the conidiophore. The conidiophores may become long and filiform in artificial media. The liberation of the spores from the pycnidium is effected in damp weather by means of distinct cirrhi, or threads, forced out through the ostiole of the pycnidium.

## INOCULATIONS

Inoculations were carried on in the greenhouse on *Castanea dentata* (Marsh) Borkh. and on a number of related species of oak—*Quercus prinus* L., *Q. minor* (Marsh) Sarg., *Q. gambelii* Nutt., *Q. lobata* Nee., *Q. texana* Buckl., *Q. virginiana* Mill., *Q. alba* L., and *Q. rubra* L.

At the time of the first inoculations small potted trees were used, and these were mostly in their dormant winter condition.

The inoculations were made by sterilizing the bark with a mercuric-chlorid solution, making an incision through the bark with a sterile scalpel, and carefully inserting a portion of the mycelium. The wound was then carefully protected by a small portion of sterile cotton. Check plants were kept of all inoculations made.

The first inoculations were made on chestnut on October 24, 1911, as no chestnut oak was then available. In seven days the inoculated twigs showed a darkened area in both directions from point of infection. After one month the twigs were entirely dead from the point of inoculation outward, and the small papillæ of the fungus were visible just beneath the epidermis. The checks healed normally.

A pure culture of the fungus was obtained from a portion of a diseased twig that was brought into the laboratory. From this culture inoculations were made on November 11, 1911, as follows:

Four inoculations on *Quercus lobata*, two by means of an incision in the bark and two by simply binding on portions of mycelium in agar with sterile cotton; three inoculations on twigs of *Castanea dentata;* and three inoculations on leaves of *Q. prinus*. One leaf of *Q. prinus* was inoculated on the upper surface through the wounded epidermis and one on the lower; on the other, the mycelium was simply spread over the unwounded surface.

An examination after one week showed inoculated twigs of *Quercus lobata* blackened for about half an inch each way from the point of inoculation; the chestnut was slightly darkened. The wounded leaves of *Q. prinus*, both inoculations and checks, were somewhat yellowed, but these subsequently recovered; the unwounded inoculated leaf was normal; and all were uninjured by the fungus. After some weeks these leaves were brought into the laboratory and careful sections made, but no trace of the mycelium could be found in the tissues.

In all, a total of over 50 inoculations were made in the greenhouse to test the susceptibility of different species of oak and to find the time when infection most readily takes place. Of these inoculations 50 per cent were effective. The twigs darkened and the leaves withered, showing the presence of the fungus. In some the infection did not extend more than a few inches from the tip, but in others the whole twig died. In but few cases, however, did the fungus make its way into and up the main body of the plant.

*Quercus gambelii* proved to be the most susceptible when inoculated, and *Q. lobata* the second; *Q. alba* and *Q. rubra* were slower in showing the effects of the fungus; while *Q. virginiana* and *Q. texana* were not affected.

In a number of cases the plant was in a dormant condition when inoculated and seemed not to be affected by the fungus, but at the leafing-out season no leaves were formed from the point of inoculation outward to the end of the branch (Pl. XXXVIII), while the other part of the plant put out leaves and grew in a normal manner. After inoculation the twig darkened slightly, but no further external development took place. No pycnidia were formed as usual, even after the growing season commenced.

The failure of part of the inoculations was probably due to the time of inoculation, as it was found that the twigs are the most susceptible when the new shoots are just coming out. Practically all the inoculations made at this time were effective, but after two weeks from the time of leafing-out the susceptibility lessened greatly, only a small percentage made from that time on having any effect.

In some cases after the dying of the tip the branch put out new shoots below and apparently overcame the injurious effect of the fungus. Inoculations from cultures of the mature stage developed somewhat slower than those from the Macrophoma stage.

The inoculations of *Quercus prinus* in the field were more conclusive. Fifty inoculations were made on May 8, 1912, and 28 of these were effective. Twenty-six were made in the usual manner by a slight incision in the bark and the inserting of a portion of the mycelium into the wound. Fifteen were made by inoculating with spores. Of the latter, 10 were made by placing the spores in the incision and 5 by puncturing the bark with a needle and spraying the injured part with spores suspended in corn-meal infusion. Four inoculations were made by binding the mycelium on the surface of the uninjured twigs. Five leaves were pricked slightly with a needle and sprayed with the spores—one on both upper and lower surface, two on the upper surface only, and three on the lower only. Checks of both leaves and twigs were treated in the same manner. The leaves all healed normally and were not affected by the fungus. Three of the twigs that were sprayed with spores withered and died, while the two others healed normally. Four of the twigs inoculated with spores by a slit in the bark withered from the point of infection out to the tip; the others were uninjured by the fungus and put out new leaves and shoots. Of the 26 twigs inoculated with mycelium on wounds, 21 showed the effects of the fungus, most of them dying completely from point of inoculation outward; those unwounded showed no effects whatever but grew in a normal manner. The inoculations were made partly on small saplings and partly on the small branches of larger trees. The largest sapling which died com-

pletely was about 8 feet high and the main trunk about 1½ inches in diameter. After two weeks the ends of the twigs withered and the leaves dried up. The twigs showed the darkening of the cambium for a distance of 6 inches from the tip. Sections across the twig also showed pustules of the fungus just beneath the bark. After three months the pycnidia had broken completely through the bark, spores of both types being present in the pycnidium. On June 1, 1912, 20 other inoculations were made in the field by the wounding of the bark and inserting a portion of mycelium. Checks were treated in like manner. Of these only 7 were effective, as the twigs were by that time older and possibly more resistant. In no case were there any large limbs killed, only the small branches and tips.

## CULTURE WORK

The fungus grows well in culture, but does not fruit readily, and then only on solid media. Fresh twigs of *Quercus alba* and *Q. prinus* were brought in from the field and sterilized by wiping with mercuric-chlorid solution and rinsing with distilled water. The bark was then pricked in several places, and portions of agar containing mycelium were spread over these portions. These were then put in test tubes with sufficient moisture. In one week discolored areas appeared on the twigs, and in three weeks the small black pustules of the fungus appeared. On examination these proved to be the Macrophoma stage.

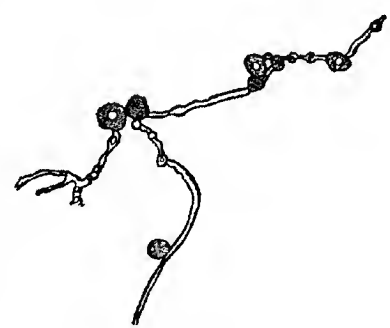


FIG. 3.—*Diplodia longispora*: Sclerotial bodies formed in artificial media.

Twigs of the same species were also used, sterilizing them by the use of the autoclave. The growth on these was almost entirely superficial, the mycelium completely covering the twigs in a grayish green, felty mass. Occasional humps or tufts of mycelium were present in which a few pycnidia containing spores of the Macrophoma type were found. After six months no further development had taken place. As a medium the autoclaved twigs proved to be much inferior to the unheated twigs.

Of the agars corn meal and prune gave the best vegetative growth and were used to the exclusion of others in securing pure cultures and in germination studies. Portions of the mycelium were transferred to corn-meal flasks or other solid media to secure the formation of pycnidia.

A number of different kinds of media were used: Potato, prune, beef, and corn-meal agars, −15; potato and beef agars, +15; corn-meal and

prune agars, +11, Fuller's scale; Raulin's fluid, malt, and string-bean agars; and cylinders of Irish potato, sweet potato, parsnip, and carrot, banana, orange, prune, and apple.

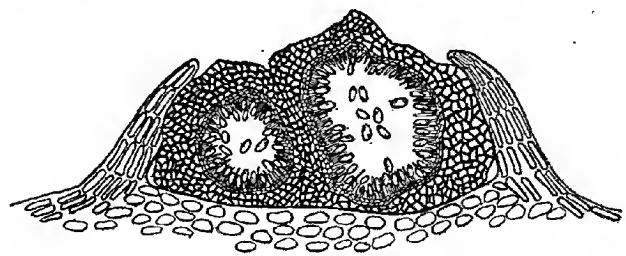


FIG. 4.—*Diplodia longispora:* A section showing grouping of pycnidia.

The Irish potato and the sweet potato gave the best results for the vegetables. The fruits gave an abundance of mycelial growth, but few pycnidia. In several media, especially apple, peculiar sclerotial bodies (fig. 3) were formed in abundance. An extremely acid or extremely alkaline medium was not as satisfactory as a nearly neutral one, and starchy media in general gave the best results. On all artificial media which produced pycnidia, a dense stroma was produced and the spores were borne in locules in the stroma. This is not the case on the host, where, while the pycnidia are usually grouped (fig. 4), a typical stroma is never present. On all media the colonies are at first hyaline, later becoming grayish green, and finally almost black.

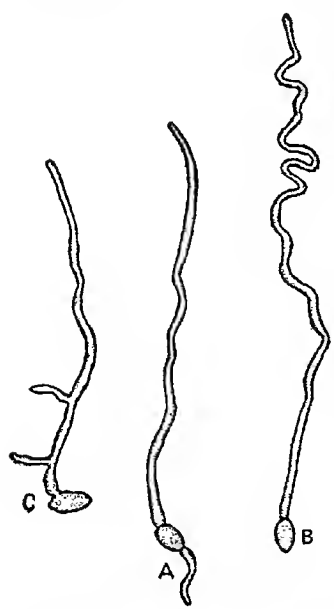



FIG. 5.—*Diplodia longispora:* Types of germination. *A*, *B*, Germ tubes from end of spore; *C*, germ tube from side of spore.

## GERMINATION STUDIES

The spores germinate readily in distilled water, corn-meal infusion, Raulin's fluid, and corn-meal, prune, or potato agar. If a diseased twig is placed in a damp chamber many spores will germinate inside the pycnidium. When placed in a liquid medium without being subjected previously to a moist atmosphere, the time varies from three to six hours.

Usually the germ tubes are sent out from the long axis of the spores (fig. 5, *A* and *B*) and occasionally from the sides (fig. 5, *C*). As many as six tubes have been observed from a single spore.

At first the tubes are nonseptate, but the cross walls gradually begin to appear in from two to five days from time of germination. The hyphæ show a marked tendency to coalesce (fig. 6), and often unite to form meshes. Soon after the formation of septæ the mycelium begins to darken, taking on a grayish green hue. The hyphæ become constricted, and peculiar chlamydosporelike bodies are formed (fig. 7) intercalary in the hyphæ. When a number of spores are sown at one time, some of them undergo a further development, instead of germinating as above described. The spore turns a dark olive brown in color, and a central, transverse septum is formed. Occasionally two septæ are present (fig. 2, *C*), but this is not typical.

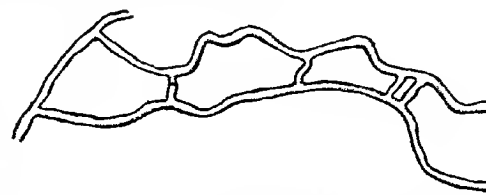


FIG. 6.—*Diplodia longispora:* A portion of mycelium showing the coalescing of the hyphæ.

FIG. 7.—*Diplodia longispora:* A portion of mycelium with chlamydosporelike bodies.

## DETERMINATION OF THE FUNGUS

In order to determine definitely whether the Macrophoma and Diplodia types of spores were really stages in the life history of the same fungus, a number of single spores of each were planted in agar plates, and carefully marked colonies of each from single spores were then transferred to cornmeal flasks. Each first produced the Macrophoma stage and later the Diplodia stage. Numbers of diseased twigs were brought in from the field and carefully examined the following winter after being attacked, in the hope of finding a perfect stage, but without success. According to Saccardo, this fungus should be called a Botryodiplodia, as the pycnidia are usually grouped. However, since the characters which separate it from the genus Diplodia may be produced artificially on culture media and vary with the amount of moisture present, it seems advisable to place it in the latter genus.

A number of species of Diplodia have been described on Quercus, mostly from European countries. All of them are described either from the immature stage, or insufficient morphological characters are given for a positive identification, the spore measurements in several being absent. Only one species has been found described from America—*Diplodia longispora* C. and Ell. on *Quercus coccinea* from New Jersey. It is the

only species which is described with mature spores and in which the spore measurements are given. The morphological characters given agree very well, but, according to the measurements given, the spores are uniformly longer and narrower, being 30 to 35 by 7$\mu$ in comparison with 23 to 32 by 8 to 12$\mu$ of the species under discussion.

However, since there is much variation in this genus and since the perfect form of this fungus may eventually be found, the species herein described is referred to *Diplodia longispora* C. and Ell. While, as mentioned above, the spore measurements do not exactly agree, the variation being considered by some sufficient to warrant a new species, it was not thought desirable to add another species to the already cumbersome and much confused nomenclature of this genus. None of the species described are recorded as causing any disease of the host.

## SUMMARY

A fungus which is referred to *Diplodia longispora* C. and Ell. is the cause of a destructive twig disease of *Quercus prinus*, also of several other species of Quercus and of *Castanea dentata*.

Large trees are not killed outright, but they may eventually die as a result of the weakened condition caused by losing the young branches, and particularly the cumulative effect of the attacks of several years. Saplings are often killed outright.

Infection takes place through wounds in the bark and will not take place through an unbroken surface. The fungus does not extend into the leaves, as no mycelium is present in the leaf tissues.

## DESCRIPTION OF PLATE

PLATE XXXVIII. An oak (*Quercus gambelii*) inoculated with *Diplodia longispora* at X when dormant. No leaves developed above the point of inoculation.


X

# NEW POTATO WEEVILS FROM ANDEAN SOUTH AMERICA

By W. DWIGHT PIERCE,

*Agent and Expert, Investigations of Insects Affecting Southern Field Crops, Bureau of Entomology*

During the year 1913 a number of shipments of South American potatoes for experimental propagation by the Department of Agriculture have been intercepted by Messrs. E. R. Sasscer and H. L. Sanford, inspectors of the Federal Horticultural Board, because of more or less serious infestations by weevils. In most of the shipments the weevils were alive. Those received early in the summer were partly immature, while in later shipments they were all mature. When the material was shipped it was supposedly free of insect pests, and in fact it is quite possible to find a potato apparently whole which contains a weevil within. Mr. C. H. T. Townsend, the Entomologist of Peru, writes that the work of the weevils is often undetected until the potatoes are cooked and served on the table. It can therefore be seen how readily a shipment of South American potatoes received for planting purposes might be passed by quarantine officers and perhaps be the source of a very dangerous pest to the American potato industry.

As a result of the finding of weevils in many shipments of potatoes, the Federal Horticultural Board has taken action excluding South American potatoes from the United States. This article has therefore been prepared with the view of assisting the inspectors in their work and also to place on record descriptions of the weevils in question.

The three species of weevils so far found are very different in appearance and can be readily identified from the illustrations published herewith.

A notice of the finding of a species of weevil known as *Rhigopsidius tucumanus* Heller in potatoes shipped by Mr. W. F. Wight from points in Peru, Bolivia, and Chile has been published.[1] Since the publication of this note two other species, each representing a new genus and a new species, have been discovered.

The second species found in shipments of potatoes from Peru was obtained alive on July 9, 1913, by Mr. Sasscer in a potato sent by Mr. Wight from the mountain districts of Peru. The adult weevil was found just under the skin of the potato in a small cell which had evidently served as a feeding cell for the larva. From the material received it is judged that the larva does not bore extensively in the potato.

[1] Sasscer, E. R., and Pierce, W. Dwight. Preliminary report of the finding of a new weevil enemy of the potato tuber. Proc. Ent. Soc. Wash., v. 15, no. 3, p. 143-144, pl. 4-5. Oct. 2, 1913.

This weevil (Pl. XLI, figs. 1 and 2; and text figs. 1 and 2) forms the type of a new genus in the family Brachyrhinidæ, subfamily Entiminæ, tribe Ophryastini, to which our North American genera Ophryastes, Eupagoderes, Amydrogmus, and Tosastes belong. In Lacordaire's group "Leptopsides vrais" it is to be placed near Bastactes and Catasarcus, from both of which it differs by many characters. The descriptions which follow will serve to identify it.

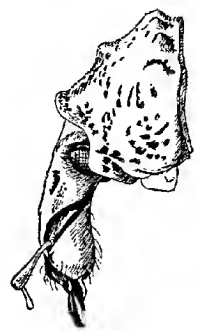


FIG. 1.—*Premnotrypes solani* Pierce: Lateral view of prothorax and beak.

PREMNOTRYPES, new genus.

Name derived from *πρέμνον* (root) and *τρυπάω* (to bore), meaning a root borer. Type of genus.—*P. solani*, n. sp.

Upper surfaces roughly sculptured throughout and closely squamose. Beak longer than head, enlarged at alæ, more or less distinctly depressed on the median line and at the sides; scrobes broadened behind and then flexed downward far from eyes; mandibles beneath not acutely toothed. Eyes vertical, elongate oval, pointed beneath. Antennæ with scape clavate, not greatly overlapping the anterior edge of the eyes; funicle 7-jointed, with first two joints elongate, the others shorter but not transverse; club elongate oval. Prothorax very tuberculate above and at sides; anterior lobes without vibrissæ, almost completely covering the eyes; base truncate, apex convex. Elytra with humeri rounded; striation irregular, with alternate intervals multituberculate. Body wingless. Thorax beneath with all parts short; mesothoracic side pieces unequal; metepimera broad. Intercoxal process broad; first two abdominal segments occupying over half the abdomen; first suture arcuate; second segment at least as long as the two following; fifth segment as long as the two preceding. Femora and tibiæ stout; tibiæ mucronate; tarsi with third joint bilobed and a little wider than the preceding joints, pubescent beneath; claws simple. The posterior tibiæ have the point of attachment of the tarsi terminal and close to the mucro. The apical surface is divided by a ridge into two unequal disks, the inner being the larger. The ridge passes just outside of the corbel.

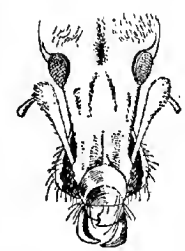


FIG. 2.—*Premnotrypes solani* Pierce: Frontal view of beak.

**Premnotrypes solani**, n. sp.

Length, 7 mm.; breadth, 3.75 mm. Color brown, with bronzy scales.

Beak longer than head and narrower than eyes, being narrowest at about the middle, where the flare of the scrobes begins to widen it. Alæ strongly flared, making apical portion of scrobes open above. Head with small tubercles above the eyes. Median line sharply defined, deepened at frontal fovea, then bifurcate to form a median ridge. The fine median line begins again on this ridge and extends to the apex. Beginning even with the front edges of the eyes the lateral impressions extend half the length of the beak. Apex of beak shining black, raised in an arcuate band, which causes the shining semielliptical nasal plate to stand obliquely. Mandibles shining black, with at least two inner teeth and with a long, shining, acute, deciduous piece with sharp inner edges. The right-hand deciduous piece has a tiny tooth on the inner edge before the middle. Antennal scrobes strongly flexed downward; scape clavate; funicle with all joints longer than wide, gradually decreasing in size toward

apex; club elongate, with the first two joints occupying over half the bulk. Head, beak, and scape densely clad with fine, silky, bronzed scales; funicle sparsely pubescent; club minutely pubescent.

Prothorax basally truncate, apically sinuate, strongly lobed over eyes, lobes without vibrissæ; coarsely punctured, finely squamose with yellowish to golden metallic scales; median line punctate, strongly impressed; surface with six basal, two discal, and four apical tubercles; widest behind middle at points of lateral basal tubercles.

Elytra at base no wider than thorax; humeri rounded; sides rounded, rough, wider than prothorax. Scutellum minute, triangular, depressed. Surface densely minutely scaly; striæ irregular, with small definite punctures; entire surface rough, but the third, fifth, and seventh intervals especially are raised by a series of small tubercles, which give the striæ a wavy direction.

Prosternum strongly arcuately emarginate, not more than one-half as long as pronotum. Anterior coxæ contiguous. Mesosternum taken up almost entirely by the coxæ, which are narrowly separated; side pieces unequal. Metasternum also short. Undersides and legs densely squamose.

Type.—Cat. No. 16689, U. S. National Museum.

The third species also belongs to a new genus quite closely related to Premnotrypes and belonging in the same tribe. Several specimens in a more or less perfect condition were found by Mr. Sanford in cells in potatoes received October 9, 1913, from Cuzco, Peru. This species breeds in a manner closely resembling that of the *Premnotrypes solani*.

This species (Pl. XLI, fig. 3; text fig. 3) may be identified from the following technical descriptions.

TRYPOPREMNON, new genus.

Name derived from τρυπάω (to bore) and πρέμνον (root), signifying a root-borer. The name is simply "Premnotrypes" reversed, because the two genera belong side by side.

*Type of genus.—T. latithorax*, new species.

Upper surfaces roughly sculptured throughout and closely squamose. Beak longer than head, enlarged at alæ, not impressed on median line except at frontal fovea and near apex; scrobes broadened behind and abruptly truncate; mandibles beneath sharply toothed. Eyes vertical, elongate oval, pointed beneath. Antennæ with scape clavate, not greatly overlapping the anterior edge of the eyes; funicle seven-jointed, joints 1 and 2 elongate, the others progressively shorter and the last three transverse, moniliform; club elongate oval. Prothorax very roughly molded; median line deeply impressed; anterior lobes without vibrissæ, almost completely covering the eyes; base truncate; apex sinuate. Elytra with humeri rounded; striation irregular, with alternate intervals rough and raised. Body wingless. Thorax beneath with all parts short; mesothoracic side pieces unequal; metepimera elongate, moderately broad. Intercoxal process broad; first two abdominal segments occupying over half the abdomen; the first suture arcuate; the second segment as long as the two following; fifth segment as long as the second. Femora and tibiæ stout; tibiæ mucronate; tarsi pubescent beneath, with third joint strongly bilobed, the lobes much wider than the preceding joints; claws simple. The posterior tibiæ have the point of attachment of the tarsi terminal and close to the mucro. The apical surface is divided by a ridge into two almost equal slanting disks, like a roof. The ridge runs directly to the middle of the corbel.

FIG. 3.—*Trypopremnon latithorax* Pierce: Lateral view of thorax and beak.

**Trypopremnon latithorax, n. sp.**

Length, 6 mm.; greatest breadth, 2.75 mm. Beak longer than head and narrower than eyes except at alæ; the dorsal squamose portion being gradually narrowed from the eyes to the apex. Alæ strongly flared, making the apical portion of the scrobes open above. Head very slightly tumid above the eyes. Median line distinct only to the frontal fovea, which is deeply depressed and very faintly indicated beyond this point. The lateral depressions on the beak are quite faint. Apex of beak shining reddish, with the nasal plate polished, ogival, and raised at apex. Mandibles shining, reddish; deciduous piece long, shining, acute, arcuate, with sharp edges and with a strong, acute, erect ventral tooth. Antennal scrobes strongly flexed downward, very much broadened and evanescent behind; scape clavate; funicle with first two joints elongate, the others progressively shorter and the last three transverse, moniliform; club elongate oval. Head, beak, and scape densely clad with fine, silky, bronzed scales; funicle sparsely pubescent; club minutely pubescent.

Prothorax basally truncate, apically sinuate, with very strong supraocular lobes, which are without vibrissæ; coarsely irregularly punctured, finely squamose with golden metallic scales; median line strongly impressed; surface very uneven with two basal and two discal elevations and with the sides very irregular, sinuate or bitumid; widest at posterior lateral tumidities.

Elytra at base narrower than thorax; humeri rounded; sides feebly convex. Scutellum triangular. Surface densely, minutely scaly; striæ irregular, with strong punctures, entire surface rough, but the third, fifth, and seventh intervals especially are raised by a series of tubercles, which give the striæ a wavy direction.

Prosternum strongly arcuately emarginate, hardly half as long as the pronotum. Anterior coxæ contiguous. Mesosternum taken up almost entirely by the coxæ, which are narrowly separated; side pieces unequal. Metasternum also short. Undersides and legs densely squamose.

Type.—Cat. No. 16690, U. S. National Museum.

Differs from *Premnotrypes solani* in the sculpturing of the beak, the shape of the scrobes and mandibles, and of the nasal plate, the absence of distinct tubercles on the head, the shape and sculpture of the prothorax, and the elytral striation. The third tarsal lobes are also much more distinct.

The weevil *Rhigopsidius tucumanus* Heller (Pl. XL) is, according to present information, more widely distributed than either of the other species. It was originally described by Heller[1] from Tucuman, Argentina, and was recorded in the note by Sasscer and Pierce,[2] quoted above,

[1] Heller, K. M. Neue Rüsselkäfer aus Central- und Südamerika. Ent. Ztg. Stettin, 1906. Bd. 67 (Heft 1), p. 7–9, pl. 1., figs. 3, 3a, and 3b.

[2] This weevil (Pl. XL) belongs to the family Psalidurida, subfamily Rhytirhininæ, tribe Rhytirhinini. The nearest North American insects are the species of the genus *Thecesternus* in the tribe Thecesternini of the same subfamily.

The following description, taken from Sasscer and Pierce (op. cit.), will identify this species.

Length, 9 mm., yellowish or purplish brown, with thickly matted vestiture of a cinereous shade mottled with black dots. Head concealed from above by prothorax and eyes, almost covered by the lateral prothoracic lobes. Beak moderately short, usually reposing in a deep pocket of the prothorax, which is posteriorly limited by the anterior coxæ. Beak medianly and laterally carinate to a cross carina between the bases of the antennal scapes. Scrobes deep and narrow from apex near tip of beak almost to eyes, then sharply deflected and broader in front of eyes. Scape stout, clavate. Funicle 7-jointed, the last joint apparently a part of the club. Club 4-jointed. Head at base sinuately impressed, with swellings above the eyes. Prothorax very irregularly sculptured but with a deep median furrow widened angularly at middle and also behind. Strial punctation deep but irregular. Intervals tumid behind. Legs stout. Tarsi with third joint not widely bilobed; tarsal claws simple. First and second abdominal segments long; third and fourth shorter than fifth.

in shipments received May 24, 1913, from Mr. Wight, who collected the material at Cuzco, Temuco, and Arequipa, Peru; Oruro, Bolivia, and Ancud or San Carlos and Castro Islands, Chile. In many instances the injury occasioned by these weevils was quite noticeable. A few of the tubers which superficially appeared to be sound were found, on being opened, to be infested with one and sometimes two larvæ or adults. Mr. Sasscer succeeded in keeping two adults alive from May 24 to September 6, during which period they fed but little and then only on foliage of potato. The injury of this species consists of tunnels throughout the potato, as shown in Plate XXXIX, and the work of the two other weevils is very similar.

## DESCRIPTION OF PLATES

PLATE XXXIX. Injury caused by potato weevils. Fig. 1.—A section of a potato from Peru, showing the larva of *Rhigopsidius tucumanus* in its burrow.

Fig. 2.—A section of a potato, showing the burrowings of *Rhigopsidius tucumanus*. The work of the two other weevils is somewhat similar.

XL. *Rhigopsidius tucumanus* Heller. Fig. 1.—Dorsal view.

Fig. 2.—Ventral view. Both views are much enlarged; natural size, 9 mm.

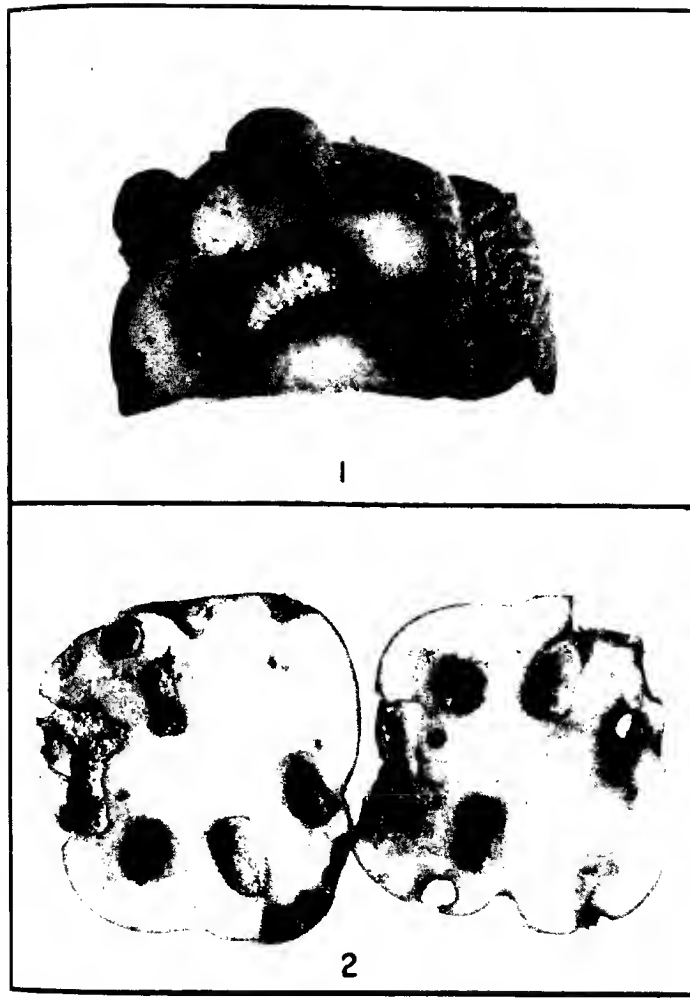
XLI. Figs. 1 and 2.—*Premnotrypes solani* Pierce (much enlarged; natural size, 7 mm.).

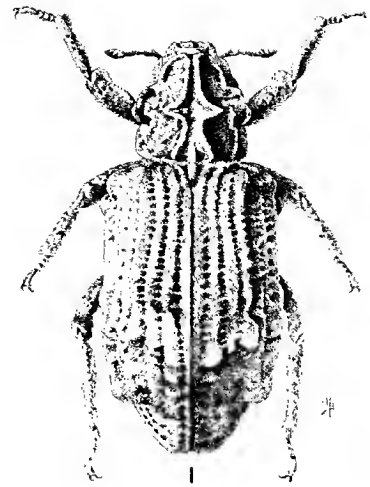
Fig. 1.—Dorsal view. In this drawing the beak, scape, and tibiæ are foreshortened, which gives an idea of even greater differences from the succeeding species than really exist.

Fig. 2.—Ventral view.

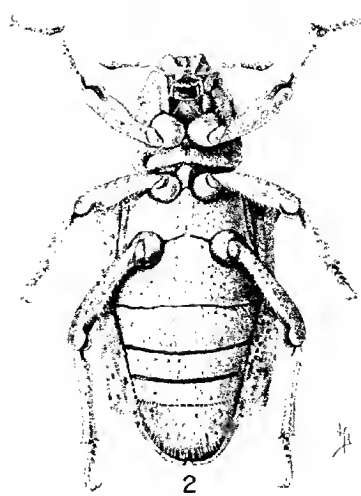
Fig. 3.—*Trypopremnon latithorax* Pierce (much enlarged; natural size, 6 mm.). Dorsal view. In this drawing the scape and the tibiæ are not foreshortened as much as in the other species. The different attitude of the beak gives a sense of greater divergence than occurs, as can be seen from the side view of the head and prothorax (see text figs. 1 and 3). The ventral view resembles very closely that of *Premnotrypes solani*.

The drawings which accompany this article were made by Mr. Harry B. Bradford.

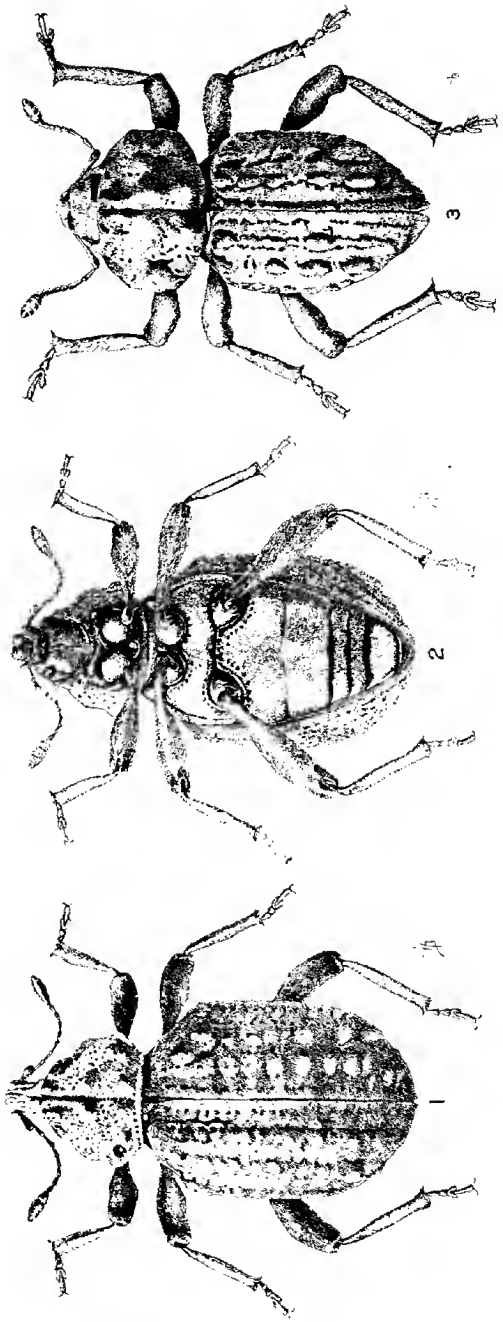

1
2



1



2


3
2
1

# AN UNDESCRIBED SPECIES OF GYMNOSPORANGIUM FROM JAPAN

By W. H. LONG,

*Forest Pathologist, Investigations in Forest Pathology, Bureau of Plant Industry*

## INTRODUCTION

In the Annual Report of the Connecticut Agricultural Experiment Station for 1912 (pt. 5, p. 350), Dr. Clinton reports the introduction of *Gymnosporangium japonicum* Syd. on *Juniperus chinensis*. The rust was found on both stems and leaves of a form known as *J. compacta*, while on a seedling of *J. chinensis* called *J. virginalis* the rust occurred only on the leaves. The plants showing rust only on the leaves were planted in an isolated place. The following spring they were found to be free from rust.

Through the kindness of Dr. Perley Spaulding the writer was able to examine some of the infected material from Dr. Clinton's herbarium containing both types of the rust. The rust on the woody stems seems to be *Gymnosporangium japonicum* Syd., but that on the leaves or young twigs differs in most of its microscopic and macroscopic characters from *G. japonicum*. According to the report, the rust on the leaves or young twigs is apparently an annual, while the other, *G. japonicum*, is a perennial; one is found on the leaves and green twigs, the other on the woody stems; one causes no deformation of the host, the other produces fusiform enlargements 4 cm. in length or longer. The microscopic characters of the two differ as widely as the gross characters mentioned above.

The writer has found in most species of Gymnosporangium three types of teliospores in the same sorus. One type has very thick colored walls; one, moderately thick colored walls; and the third, thin and colorless walls. These three types usually differ from each other also in shape and size of the spore as a whole or in the individual cells of each spore. Constant specific characters may occur in one type, often in the thin colorless-walled spores, while they are absent in the other two types or are not so pronounced. For this reason the characters of at least the two extreme types of spores should be given for each species under discussion. In the following descriptions the two extreme types are fully described for two of the species and the three types for the third one. As a matter of convenience in comparing the three species brief descriptions of *G. japonicum* and *G. haraeanum* are also given.

## DESCRIPTION OF SPECIES OF GYMNOSPORANGIUM

**Gymnosporangium chinensis, n. sp.**

Æcia unknown.

Telia epiphyllous or caulicolous, appearing on the very small green twigs between the leaves, not causing a fasciation of the young shoots; scattered; usually hemispheric; about 1 mm. in diameter; hazel in color when desiccated.

Teliospores 2-celled; spores with colored walls, oval to broadly ellipsoid, 19 to 22 by 35 to 40 $\mu$ (average for 10 spores, 21 by 36.7 $\mu$), slightly but plainly constricted at septum. The two cells are usually subequal; spores rounded at both ends, walls thin, about 1 to 1.5 $\mu$, pedicel cylindric; pores, one to two in each cell near septum, or rarely only one in upper cell and apical.

Spores with thin colorless walls, ellipsoid, 17 to 19 by 47 to 52 $\mu$ (average for 10 spores, 18 by 49 $\mu$), plainly constricted at septum. The two cells are unequal, the lower being from 3 to 7 $\mu$ longer than the corresponding upper cell; apical cell rounded or only slightly narrowed toward apex, lower narrowed toward base; wall thin, colorless, about 1 $\mu$ thick; pores, one to two in each cell near septum, or usually only one in upper cell and apical.

Host plant.—On *Juniperus chinensis* in the Elm City Nursery, Westville, Conn., March 28, 1911, on stock just imported from Japan. In same packet with *Gymnosporangium japonicum* on the same host. From the herbarium of Dr. G. P. Clinton.

**Gymnosporangium japonicum** Syd.

Telia caulicolous on fusiform enlargements, 4 cm. or more long, of the woody stems, irregular tongue or wedge shaped, about 3 mm. or more long, often in rows.

Teliospores 2-celled, occasionally 3-celled; spores with thick colored walls, ellipsoid, 22 to 24 by 48 to 63 $\mu$ (average size for 10 spores 22 by 54.7 $\mu$), cells subequal or lower longer and more narrowed at base, not constricted at septum, narrowed at both ends; walls 1.5 to 2 $\mu$ thick, pores near septum, two in each cell.

Spores with thin colorless walls, elliptic fusiform to linear oblong, 16 to 19 by 57 to 70 $\mu$ (average for 10 spores 16.8 by 65 $\mu$), walls 1 $\mu$ thick, not constricted at septum; pores, two in each cell near septum.

Host plant.—On *Juniperus chinensis* from Japan.

**Gymnosporangium haraeanum** Syd.

(Sydow, H., and Sydow, P. Novae fungorum species—VIII. Ann. Mycol., v. 10, no. 4, p. 405, 1912.)

Telia epiphyllous or caulicolous on the very small green twigs, not causing a fasciation of the young shoots; scattered; hemispheric to short conic; one-half to 1 mm. in size.

Teliospores 2-celled; spores with very thick colored walls, ellipsoid, 25 to 28 by 35 to 44 $\mu$ (average size for 10 spores 25.7 by 39 $\mu$), not or but very slightly constricted at the septum; spores rounded or somewhat narrowed at both ends; the two cells subequal or the lower often larger and more narrowed toward the base than the upper one; walls very thick, 3 to 4 $\mu$; pores, two in each cell near septum; pedicel cylindrical.

Spores with walls moderately thick and colored, elliptic oblong, 22 to 26 by 48 to 57 $\mu$ (average for 10 spores 23.6 by 52 $\mu$), not or but slightly constricted at septum; spores usually much narrowed at both ends; upper cell often with a mammillate apex; lower cell often longer than upper; walls 2.5 to 3 $\mu$ thick; pores, two in each cell near septum.

Spores with walls thin and colorless, oblong to oblong fusiform, 16 to 19 by 48 to 57 $\mu$ (average size for 10 spores 17 by 51 $\mu$), rarely constricted at septum; cells subequal, rounded or narrowed at both ends; pores, two in each cell near septum; walls about 1 $\mu$ thick.

Host plant.—On *Juniperus chinensis* from Japan.[1]

[1] This description was made from a portion of the type material which Dr. Sydow kindly sent to the writer.

The three types of spores are described in full, and their diagnostic character can readily he seen when *Gymnosporangium haraeanum* is compared with the other two species given in this paper. In *Gymnosporangium japonicum* and *G. chinensis* the spores with thick and moderately thick colored walls for each species are so similar that the two kinds are described as one; therefore, only two types of spores, thick and thin walled, are described for each of these two species. *G. chinensis* and *G. haraeanum* are so closely related that the writer would not publish the former as a new species until he had examined the type material of the latter. After a careful examination, however, the conclusion was reached that the two were distinct, as they differ in certain fundamental microscopic characters. These differences are shown in the description given of each species. The most marked difference between these two species is the position of the germ pores in the colorless thin-walled teliospores. In *G. chinesis* they are plainly apical in the upper cell, while in *G. haraeanum* they are just as certainly situated only at the septum in both cells.

According to Dr. Clinton, the telia of *Gymnosporangium chinensis* occur on the leaves, hut in the very meager herbarium material examined by the writer they arose between the leaves rather than on them. The telia are therefore stated in the above description to be either caulicolous or epiphyllous.

The three types of spores mentioned in the above descriptions are usually more evident in herbarium material than in fresh, as the obstructing colored contents of the spores fade in drying, thus permitting a clearer view of the spore walls.

The value of taking into consideration at least two types of spores, the thick and thin walled ones, is very evident when the corresponding kinds for each species are compared. For instance, the oval thick-walled spores of *Gymnosporangium chinensis*, with equal cells rounded at hoth ends, are in marked contrast to the ellipsoid, thick-walled spores of *G. japonicum*, with unequal cells sharply contracted at both ends; while the long, narrow, lincar-oblong, thin-walled spores, with equal cells of *G. japonicum*, are very different from the shorter thin-walled spores, with unequal cells of *G. chinensis*. Again, many of the thick-walled spores of *G. japonicum* are so sharply attenuated at both ends that they become trapezoid in shape, while the apical cells often have a distinctly mammillated apex. Neither of these characters is present in the thick-walled spores of *G. chinensis*.

Through the kindness of Dr. Shirai the writer has been ahle to examine some of the material of *Gymnosporangium japonicum* collected in 1900. It was prohahly a part of the material used hy him in his inoculation experiments with this species.[1] The specimens sent consist of two

[1] Shirai, M. Über den genetischen Zusammenhang zwischen Rostelia koreaensis P. Henn. und Gymnosporangium japonicum Sydow. Ztschr. Pflanzenkrank., Bd. 10, Heft 1, p. 1–5, pl. 1–2, 1900.

infected branches. One lesion is on a woody stem 6 mm. in diameter; the other is on a much younger branch 1.5 mm. in diameter. No telia were found on the leaves or very young twigs. The telia and teliospores were similar to those found on the woody stems of the imported *Juniperus chinensis* from Connecticut, but had nothing in common with the telia of *G. chinensis*, which were found on the very young twigs and leaves of this imported juniper.

ADDITIONAL COPIES of this publication may be procured from the SUPERINTENDENT OF DOCUMENTS, Government Printing Office, Washington, D. C., at 25 cents per copy

Subscription, per year, 12 numbers - - $2.50